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How can miscanthus be integrated most efficiently into agricultural production systems?

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Content

List of Acronyms

A	*annum* (Latin = year)
ADF	acid detergent fibre
ADL	acid detergent lignin
a.s.l.	above sea level
C	collar
C	carbon
C:N ratio	carbon : nitrogen ratio
CH_4	methane
Cm	centimetre
cm^3	cubic centimetre
D	depth
DM	dry matter
DMC	dry matter content
DMC_{silage}	dry matter content of silage
DMY	dry matter yield
EMI	European Miscanthus Improvement
e.g.	*exempli gratia* (Latin = for example)
FIA	flow injection analyser
FM	fresh matter
G	gram
HD	harvest date
GC	gas chromatograph
GW_{el}	gigawatt electric

Ha	hectare
hPa	hecto Pascal
H	hour
HPLC	high performance liquid chromatography
i.e.	*id est* (Latin = that is)
K	Potassium
Kg	kilogramme
LAZBW	Landwirtschaftliches Zentrum Baden-Württemberg
LTZ	Landwirtschaftliches Technologiezentrum Augustenberg
M	metre
m^2	square metre
m^3	cubic metre
Mm	millimetre
Mxg	*Miscanthus × giganteus*
MSac	*Miscanthus sacchariflorus*
MSin	*Miscanthus sinensis*
MY	methane hectare yield
N	nitrogen
NDF	neutral detergent fibre
(N)m^3	(norm) cubic metre
(N)ml	(norm) millilitre
N_{min}	soil mineral nitrogen
No	number
N_2O	Nitrous oxide

NO^{3-}	nitrate
Ns	non significant
oDM	organic dry matter
OPM	Optimisc (Optimizing Miscanthus Biomass Production)
P	Phosphorus
R	rhizome
Rpm	rounds per minute
R+C	rhizome + collar
SMY	substrate-specific methane yield
T	ton
VDLUFA	Association of German Agricultural Analytic and Research Institutes

Abstract

The demand for biomass is increasing steadily, as fossil resources are gradually being replaced by biomass within the context of a developing bioeconomy. Plant-based feedstocks currently used for this replacement virtually all come from annual crops. However, perennial crops such as miscanthus are expected to be more environmentally benign due to their generally low-input requirements and high yield potential.

Despite these advantages, the current cultivation area of miscanthus in Europe is quite low. One reason for this is that the cultivation and utilization of miscanthus faces several challenges. For example, the most common propagation method via rhizomes is very labour-intensive and thus expensive, leading to high establishment costs. Seed propagation is a promising option to reduce costs, but is not suitable for sterile genotypes. Another challenge to be overcome is the problem of re-integrating former miscanthus fields into crop rotations. The crop following miscanthus needs to be highly competitive in order not to be impaired by resprouting miscanthus shoots and thus able to achieve high yields. Additionally, there is only little information available on the effect of miscanthus cultivation and its subsequent removal on soil N content. This information is however crucial, for example to avoid environmental problems being caused by a potential nitrogen leaching after a miscanthus removal. If miscanthus is to be utilized as a biogas substrate, there are further challenges to be overcome. Firstly, the optimal harvest date needs to be defined with regard to the methane hectare yield and resilience of the crop to green cutting. Secondly, as a continuous supply of biomass throughout the year is necessary, ensiling will become a relevant topic. However, information is still required on the optimal harvest date to achieve a sufficient silage quality and the effects of ensiling on methane hectare yield. Finally, the suitability of miscanthus for biogas production is also influenced by biomass quality such as the proportions of leaf and stem. This has already been established for miscanthus utilization in combustion but has not yet been sufficiently investigated for anaerobic digestion.

In summary, there are a number of uncertainties involved in miscanthus establishment, removal and utilization, which currently hamper its integration into agricultural production systems. From a bioeconomic point of view, this integration needs to be conducted as efficiently as possible in terms of nutrient-use, environmental and land-use efficiency. The aim of this study was to contribute to the filling of these knowledge gaps. To achieve this aim, the following

research questions were investigated: 1) Is vegetative propagation of miscanthus via collar fragments a viable alternative to current propagation methods? 2) Which subsequent crop most efficiently suppresses resprouting miscanthus after its removal? 3) What happens to the soil nitrogen content after a miscanthus removal? 4) When is the most suitable harvest window for an autumn green cut of miscanthus to be used as biogas substrate? This can be defined as the optimal date for silage quality and high methane hectare yields while still ensuring long-term productivity of the crop. 5) What are the optimal genotype characteristics for the achievement of high methane hectare yields?

To answer these research questions, several miscanthus field trials and laboratory experiments were conducted: a novel propagation method was tested; the re-integration of miscanthus fields into a crop rotation was analysed; and the effect of genotype, harvest date and ensiling on the digestibility and methane hectare yield was investigated.

The results illustrate some possibilities of improving the nutrient-use, environmental and land-use efficiency of miscanthus biomass production along its supply chain: It was shown that miscanthus propagation via collars is feasible and a promising alternative to rhizome propagation, as the multiplication rate of collars is comparable to that of rhizome propagation. As the harvesting of collars is likely to be less labour-intensive and is less destructive for the mother field than rhizome propagation, this method is more favourable for both economic and ecological reasons. The re-integration of miscanthus into crop rotations revealed maize to be a suitable crop after miscanthus, as it coped with the prevailing soil conditions and suppressed resprouting miscanthus efficiently, resulting in satisfactory yields. The soil mineral nitrogen (N_{min}) content was found to increase during the vegetation period following a miscanthus removal, but was generally on a low level (average: 17.3 kg N_{min} ha^{-1}). Additionally, it was found that, in Germany, miscanthus should be harvested in mid-October to maximize methane yields and nutrient recycling but minimize yield reduction. In addition, silage quality was best when miscanthus was harvested on this date. As leaf proportion correlated positively with substrate-specific methane yield (SMY) and thus genotypes with a higher leaf proportion were found to have a higher SMY, methane hectare yields could be increased even further by using genotypes with a high leaf proportion.

In summary, the approaches developed in this study allow to considerably improve the ecological and economic performance of miscanthus production by increasing nutrient-use, environmental impact and land-use, and thus simplifying implementation into practice.

Zusammenfassung

Im Rahmen einer wachsenden Bioökonomie steigt der Biomassebedarf stetig an, da fossile Ressourcen nach und nach durch biobasierte ersetzt werden. Die dafür benötigte Biomasse wird derzeit überwiegend mittels einjähriger Kulturpflanzen gedeckt. Mehrjährige Kulturpflanzen, wie zum Beispiel Miscanthus, bilden aufgrund ihres geringen Ressourcenbedarfs bei einem gleichzeitig hohen Ertragspotential eine interessante, umweltverträglichere Alternative.

Trotz dieser Vorteile ist die Anbaufläche von Miscanthus in Europa derzeit gering. Ein Grund hierfür ist, dass der Anbau und die Nutzung von Miscanthus verschiedene Herausforderungen mit sich bringen. So ist zum Beispiel die übliche Vermehrungsmethode über Rhizome sehr arbeitsintensiv, was zu hohen Etablierungskosten führt. Eine Vermehrung über Samen ist zwar im Hinblick auf eine Kostenreduktion vielversprechend, aber diese Methode ist für sterile Genotypen nicht anwendbar. Eine weitere Herausforderung, bei der Integration von Miscanthus in die Praxis, ist die Wiedereingliederung früherer Miscanthusfelder in eine Fruchtfolge. Die Folgefrucht sollte den wiederaustreibenden Miscanthus effektiv unterdrücken und gleichzeitig einen möglichst hohen Ertrag generieren. Darüber hinaus gibt es derzeit nur wenig Information darüber, wie sich der Stickstoffgehalt im Boden nach einem Miscanthusanbau und anschließendem -umbruch verhält. Diese Information ist aber entscheidend, um negative Umweltwirkungen, beispielsweise durch Nitratauswaschung, zu vermeiden.

Weitere Herausforderungen ergeben sich bei der Nutzung der Miscanthusbiomasse, beispielsweise als Biogassubstrat. In einem ersten Schritt muss der beste Erntezeitpunkt im Hinblick auf den Methanhektarertrag und der Widerstandsfähigkeit der Kultur gegenüber einem Grünschnitt bestimmt werden. Des Weiteren ist die Silierung ein wichtiges Thema, da eine kontinuierliche Substratverfügbarkeit über das Jahr gewährleistet sein muss. Jedoch fehlen noch Informationen darüber, wann der optimale Erntezeitpunkt ist, um eine ausreichende Silagequalität zu erzielen, und wie sich die Silierung auf den Methanhektarertrag auswirkt. Darüber hinaus wird die Eignung von Miscanthus für die Biogasproduktion auch durch die Biomassequalität, wie beispielsweise das Blatt-/Stängelverhältnis, beeinflusst. Ähnliches wurde bereits für Nutzung von Miscanthus für die Verbrennung gezeigt, für die Biogasproduktion wurde diese jedoch noch nicht hinreichend untersucht.

Zusammengefasst gibt es im Bereich der Etablierung, des Umbruches sowie der Nutzung von Miscanthus mehrere Barrieren, die derzeit eine Integration in landwirtschaftliche

Produktionssysteme erschweren. Das Ziel dieser Studie war es, die identifizierten Hindernisse zu überwinden und dabei die Integration von Miscanthus in Bezug auf die Nährstoffnutzung, Umweltwirkung und die Landnutzung so effizient wie möglich zu gestalten. Um dieses Ziel zu erreichen, wurden folgende Forschungsfragen aufgestellt: 1) Ist die vegetative Vermehrung von Miscanthus über Collars eine praktikable Alternative im Vergleich zu vorhandenen Vermehrungsmethoden? 2) Welche Folgekultur unterdrückt am effizientesten wideraustreibenden Miscanthus nach seinem Umbruch? 3) Wie verhält sich der Stickstoffgehalt im Boden nach einem Miscanthusumbruch? 4) Wann ist der optimale Erntezeitpunkt für einen Grünschnitt von Miscanthus, wenn dieser als Biogassubstrat genutzt wird? Dieser Erntezeitpunkt umfasst dabei die Silagequalität, hohe Methanhektarerträge und soll gleichzeitig die langfristige Produktivität von Miscanthus sicherstellen. 5) Was sind die optimalen Eigenschaften eines Miscanthus-Genotyps um hohe Methanhektarerträge zu erzielen?

Um diese Fragen beantworten zu können, wurden mehrere Feld- und Laborversuche durchgeführt: So wurde eine neue Vermehrungsmethode getestet; die Wiedereingliederung von Miscanthusfeldern in die Fruchtfolge wurde untersucht; die Wirkung verschiedener Genotypen, Erntetermine und Silierung auf die Verdaulichkeit und den Methanhektarertrag wurde analysiert.

Die Ergebnisse demonstrieren, wie die Nährstoffnutzungs-, Umweltwirkung und die Landnutzungseffizienz der Miscanthusbiomasseproduktion entlang der gesamten Versorgungskette verbessert werden können. So konnte gezeigt werden, dass die Vermehrung von Miscanthus über Collars möglich und eine vielversprechende Alternative zur Rhizomvermehrung ist. Die Vermehrungsrate ist zwar vergleichbar, die Ernte von Collars ist jedoch weniger arbeitsintensiv und destruktiv für das Mutterfeld. Aufgrund dessen hat diese Methode vielfältige ökonomische und ökologische Vorteile im Vergleich zur Rhizomvermehrung. Der Versuch, der sich mit der Wiedereingliederung von Miscanthusflächen in Fruchtfolgen beschäftigte, zeigte, dass Mais eine geeignete Folgefrucht ist. Dieser kam mit den vorherrschenden Bodenbedingungen zurecht, unterdrückte den wiederaustreibenden Miscanthus effizient und lieferte gleichzeitig hohe Biomasseerträge. Es stellte sich heraus, dass in der Vegetationsperiode nach dem Miscanthusumbruch der Bodenstickstoffgehalt (N_{min}) zwar anstieg, aber generell auf einem niedrigen Niveau lag (im Mittel 17,3 kg N_{min} ha^{-1}). Darüber hinaus wurde gezeigt, dass in Deutschland ein Erntetermin Mitte Oktober optimal ist, um die Methanerträge und die Nährstoffrückverlagerung zu maximieren und Ertragsverluste zu minimieren. Außerdem wurde aufgezeigt, dass die Silagequalität von Miscanthus an diesem Erntetermin am besten war. Des Weiteren konnte in

den Versuchen eine positive Korrelation von Blattanteil und dem substratspezifischen Methanertrag (SME) demonstriert werden. Hierbei hatten Genotypen mit einem höheren Blattanteil einen höheren SME. Basierend darauf, könnten Methanhektarerträge durch die Nutzung von Genotypen mit höheren Blattanteilen noch weiter verbesset werden.

Zusammengefasst erlauben die in dieser Studie entwickelten Ansätze das ökologische und ökonomische Leistungsverhalten der Miscanthusproduktion, durch eine Verbesserung der Nährstoffnutzung, Umweltwirkung und Landnutzung, deutlich zu steigern und dadurch die Implementierung in die Praxis zu vereinfachen.

1 General introduction

The demand for biomass is increasing steadily worldwide. One reason for that is the replacement of fossil by biobased resources within a developing bioeconomy. Bioeconomy means *"the knowledge-based production and utilization of renewable resources, in order to provide products, processes and services in all economic sectors within the context of a future-capable economic system"* (BMBF & BMEL, 2015). The various sectors, in which fossil can be replaced by renewable resources, are for example the automotive sector, building industry, chemical industry or the energy sector (BMBF & BMEL, 2015).

A good example for a well working bioeconomy is the steady increase of the renewable resources used in the energy sector. Renewable energies (mainly energy from wind, solar and biomass) have a proportion of 13.1% in Germany´s primary energy consumption (FNR, 2018). The share of renewable energies used to generate electricity has more than doubled from 2010 (15%) to 2017 (36%) (FNR, 2018). In the heat (13%) and transportation sector (5%), the proportion of renewable energies has been stagnating since 2010 (FNR, 2018). Considering the primary energy consumption, biomass is currently the most important renewable energy resource and has a proportion of about 54% within the renewable energies (FNR, 2018).

1.1 Perennial biomass crops for a growing bioeconomy

The cultivation area of biomass crops has doubled within the last 10 years in Germany and adds up to approximately 2.7 million hectares (FNR, 2018). About 90% of these cultivated crops are used as energy crops for biodiesel (31%), bioethanol (9%) and biogas production (60%) (FNR, 2018). The remaining 10% are used for industrial purposes such as industrial starch, which is a raw material for bioplastics (FNR, 2018). In Germany mainly annual crops, such as cereals (wheat, rye; 44%) or maize (45%) are used for bioethanol production. For biodiesel, rapeseed (33%) plays the most important role. For biogas production, it is also maize (69%) which is mainly used.

All mentioned crops are annual crops, which have to be established for each vegetation period again. This, however, is in a certain way unfavourable to the aim of producing environmentally benign energy crops. Lewandowski & Schmidt (2006) specified some aspects, which are important for an environmentally benign energy crop cultivation: Besides a low erosion potential and the impact on biodiversity, they mentioned a low demand for nitrogen fertilizer, plant protectants and (fossil) energy. Referring to (fossil) energy and nitrogen demand, a low

demand is preferable as then greenhouse gas emissions are minimized (Lewandowski & Schmidt, 2006), which is in turn one of the aims of bioeconomy. Therefore, Lewandowski & Schmidt (2006) conclude, that a crop is the more environmentally benign the less input, such as energy and nitrogen, is required along with a high output.

Following the results of Cosentino et al. (2018), those mentioned requirements are best fulfilled by perennial crops. Perennial crops are characterized by a high resource-use efficiency concerning water, radiation and nutrients and are therefore seen as low-input crops (Cosentino, 2018). The lower soil tillage demand in perennials reduces the risk for soil erosion and increases soil carbon sequestration (Lewandowski et al., 2003; Cosentino et al., 2018). Furthermore, as soil is covered the whole year by perennials, nutrient run-off as well as the demand for crop protection are reduced compared to annual crops (Consentino, 2018). Additionally, perennials need to be established once, but can be harvested over several years, which reduces the demand for (fossil) energy (Cosentino et al., 2018).

Examples for perennial grasses, which are currently focussed on conducting research in the USA and Europe, are switchgrass (*Panicum virgatum* L.), reed canary grass (*Phalaris arundinacea* L.) giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus spp*.) (Iqbal et al., 2015; Kiesel et al., 2017; Cosentino et al., 2018; Nocentini et al., 2018). Following Bocquého & Jacquet (2010) and Heaton et al. (2010) miscanthus can be seen as the most promising lignocellulosic biomass grass in terms of yield and environmental performance for a temperate climate, which is one reason, why the current study focuses on miscanthus.

One example for the more environmentally benign performance of perennial compared to annual grasses is shown by the study of Kiesel et al. (2017), who compared miscanthus and switchgrass with maize, which were used as biogas substrates. The study demonstrated a higher demand for (fossil) energy and a lower nitrogen-use efficiency of maize compared to the tested perennials (Kiesel et al., 2017). This resulted in a lower reduction potential in the impact categories ‘climate change’ and ‘fossil fuel depletion’ of maize compared to the perennials, which is why the authors conclude that perennials, especially miscanthus, perform more environmentally benign than annual grasses (Kiesel et al., 2017). Cosentino et al. (2018) go even further and characterize perennial grasses as ‘ideal biomass crops’, due to their high yielding, high resource-use efficiency and their ability to be grown under difficult soil conditions (for example marginal and contaminated soils).

The perennial crop miscanthus has its origin in South-East Asia and was introduced to Europe in 1935 by Aksel Olsen (Linde-Laursen, 1993). The most used genotypes for breeding-

programs are the species *Miscanthus sinensis*, *M. sacchariflorus* and *M. floridulus* (Lewandowski et al., 2016; Clifton-Brown et al., 2017). However, the most commonly cultivated and so far only commercially available genotype is *Miscanthus x giganteus* (*Mxg*) (Clifton-Brown et al., 2015). As a perennial crop, miscanthus can be harvested annually over a period of up to twenty years after a one-year establishment phase at the beginning of its lifetime (Christian et al., 2008; McCalmont et al., 2017). In Germany, common yields of *Mxg* range between 12.6 - 22 t dry matter (DM) ha^{-1} for a brown harvest, which is conducted after winter (Gauder et al., 2012; Iqbal et al., 2015; Schmidt et al., 2018) and between 18.9 - 27 t DM ha^{-1} for a green harvest, which is conducted in autumn (Kiesel et al., 2017; Schmidt et al., 2018).

Miscanthus has a high nutrient-use efficiency and a high absorption ability for nutrients due to its *"deep and extensive rooting system"* (Cadoux et al., 2012). Its nutrient recycling back to rhizomes and its leaf fall before harvest are also reasons why miscanthus has a low demand for nitrogen fertilization and a high nutrient-use efficiency (Lewandowski & Schmidt, 2006; Cadoux et al., 2012). Additionally, McCalmont et al. (2017) reported that under miscanthus cultivation soil carbon increases with 0.7 - 2.2 t C4-C ha^{-1} y^{-1}. Furthermore, miscanthus has an energy output/input ratio of about 47.3, which exceeds the ratio of annual crops (maize, rapeseed) by almost ten times (Felten et al., 2013).

1.2 Inefficiencies in miscanthus cultivation

The current cultivation area of perennials in Europe is still quite low, despite all mentioned advantages of perennial crops in general and particularly for miscanthus. In the EU-28 states, only about 43,800 ha of arable land is cultivated with perennial energy crops exclusively grown for renewable energy purposes, such as miscanthus or reed canary grass (Eurostat, 2013; Cosentino, 2018). To compare, about 60% of the agricultural land is arable land, which is cultivated with annual crops such as cereals and about 32% is grown with permanent grassland and meadow (Eurostat, 2013).

Following the results of Don et al. (2012) miscanthus is, besides reed canary grass, one of the main perennial energy crops grown in Europe. In this context, Lewandowski et al. (2016) reported of about 19,000 ha of miscanthus cultivation area. While reed canary grass is mainly cultivated in northern Europe, especially in Finland and Sweden, miscanthus is grown across whole Europe (temperate zones), for example in the UK, Germany and Spain or Italy (Don et al., 2012), which is a further reason, why miscanthus was chosen for the current study.

However, the question arises, why the cultivation area of miscanthus is so low in comparison to other, annual crops. One major barrier for a larger cultivation rate are the high establishment costs of miscanthus (Sherrington et al., 2008; Witzel et al., 2016; Clifton-Brown et al., 2017). Referring to Witzel et al. (2016) the total establishment costs (including labour and machine costs) amount to 2,575 € ha^{-1}, whereof 80% account for rhizomes.

Another possible reason for the low cultivation area of miscanthus might be the lack of established markets for its biomass (Sherrington et al., 2008; Lewandowski et al., 2016; Witzel & Finger, 2016; Clifton-Brown et al., 2017). This leads to the so-called 'chicken-and-egg' problem: farmers will not grow perennial energy crops, because there are no existing markets for biomass (Sherrington et al., 2008). On the other hand, potential end-users will make little effort to develop technologies for those markets if biomass supply is limited and unsteady (Sherrington et al., 2008).

There are several utilization pathways for miscanthus biomass, for example energetic pathways as combustion (Iqbal & Lewandowski, 2016), ethanol production (van der Weijde et al., 2017) or anaerobic digestion (Mayer et al. 2014; Kiesel & Lewandowski, 2017) but also material utilization options such as lightweight concrete (Pude et al., 2005). Depending on the utilization pathway, miscanthus has to be harvested 'green', which means before winter or 'brown', after winter. The terms 'green' and 'brown' refer in this case to the lignin and water content in the biomass: while water content decreases, lignin content increases with a later harvest date (Kiesel & Lewandowski, 2017). High lignin contents lower the efficiency of fermentation processes (van der Weijde et al., 2017) as it reduces the biodegradability of the biomass (Fernandes et al., 2009; Cossel et al., 2018). High lignin contents, however, are desirable for combustion, due to its high heating value (Lewandowski & Kicherer, 1997) and also for lightweight concretes as it serves as water barrier (Vo & Navard, 2016).

If miscanthus is used for one of the mentioned pathways, it is important, from a bioeconomic point of view, that it is used in an (resource-) efficient way (BMBF & BMEL, 2015). Efficiency means in general the proportion of a value-based output to a value-based input (Wöhe & Döring, 2010). An increase in efficiency can be achieved either by producing the same output with less input or a higher output with the same input.

Lewandowski et al. (2019) emphasised the importance of resource-use efficiency over the whole value chain, from biomass supply to use. They specified that *"resource-use efficiency can best be achieved when the various process steps in a value chain are harmonized"* (Lewandowski et al., 2019). As an example, they mentioned the biomass quality, which should

be tailored to the needs of the later utilization pathway, so that with no further input a maximised output can be achieved (Lewandowski et al., 2019). This way of thinking should be also applied to the whole miscanthus cultivation, from its establishment to its removal and utilization. But where in the miscanthus cultivation chain are possibilities to increase efficiency?

Currently, several propagation methods are existing for miscanthus, which were summarized by Xue et al. (2015), who listed all strengths and weaknesses. To propagate miscanthus, different aerial and belowground plant parts can be used. One method to propagate miscanthus is by seeds, which is not only the most promising but probably also the most efficient one at present, due to its high multiplication rate (1:1.172; Xue et al., 2015; Clifton-Brown et al., 2017). Propagation by seeds brings up some challenges, however. Currently, the most common used miscanthus genotype is *Mxg*, which is triploid and thus not able to build germinable seeds, for which reason seed propagation is unsuitable for this genotype (Xue et al., 2015). Additionally, genotypes producing seeds are feared for their potential invasiveness, which can lead to high environmental costs (Raghu et al., 2006). As a consequence, seed propagation is not feasible for every genotype and also the issue of invasiveness has to be investigated further.

Another possibility to propagate miscanthus is by micropropagation. Micropropagation has also high multiplication rates (1:960) and enables a diseases-free plant production (Xue et al., 2015). However, as it requires a high amount of labour work, and is thus costly, this method is rarely used as common propagation method (Xue et al., 2015).

Due to the weaknesses of the mentioned two propagation pathways, rhizome propagation is currently still the most common propagation method (Xue et al., 2015). The advantage of this method is that farmers can use their own plant material for propagation (Xue et al., 2015). However, to harvest the rhizomes from soil, machines for soil tillage are necessary, which implicate a destroyed mother field. This, however, decreases land-use efficiency: despite new fields can be established with the harvested rhizomes, the mother field is destructed and the regrowth of remaining rhizomes needs some time.

Stem- or rhizome-derived plants are additional possibilities to propagate miscanthus. For this, plantlets grown in the greenhouse are transplanted to the field. The advantage of this method is a high survival potential of miscanthus (Xue et al., 2015). The high amount of labour and energy input required to grow the plantlets in greenhouses (Xue et al. 2015) also reveal low efficiencies for those methods.

Another very important aspect in terms of efficiency is the yield: It is crucial for the decision, which crop should follow miscanthus after its removal. This needs to be a high-yielding crop to ensure a high use efficiency of the agricultural land. However, the subsequent crop after miscanthus should also be efficient in supressing the resprouting miscanthus: Miscanthus is a rhizomatous crop and therefore it is in this context similar to *Elymus repens* L. (couch grass). Couch grass is a well-known weed in arable farming as it can hardly be removed and leads to high yield losses due to its competitiveness by its resprouting rhizomes (Ringselle et al., 2015). Effective strategies to remove *Elymus repens* in agricultural practice are the application of glyphosate, soil tillage or cover crops (Ringselle et al., 2015). The application of glyphosate is currently highly controversially debated, as negative environmental effects are feared and as a result, the future usage of glyphosate is presently uncertain. Despite glyphosate is a disputed herbicide, it is quite effective in controlling rhizomatous weeds. If it was forbidden, resprouting *Elymus repens* should be either removed by crop competition or crop management. The same applies to miscanthus: It is resprouting through its rhizomes and thus may impair the subsequent crop leading to lower yields, which is why the resprouting of miscanthus has to be reduced. In the event, glyphosate is forbidden, miscanthus has to be controlled either by crop competition or management measures. Crop competition requires highly competitive plants, which are able to suppress the resprouting miscanthus successfully and still achieve high yields, which is important to hit a high land-use efficiency. Crop management, such as soil tillage, could also be a possibility to suppress resprouting miscanthus. However, frequently conducted soil tillage may lead to soil erosion and is connected with a high fuel consumption. This in turn decreases the energy input/output ratio on a per hectare base.

Connected to the topic, which subsequent crop should be grown, also the topic about nitrogen dynamics in soil after a miscanthus removal becomes relevant. In this context, two scenarios are possible: soil nitrogen content after a removal of the perennial crop miscanthus either behaves similar to removed permanent grassland, which means it increases due to a mineralization of plant material and soil organic matter. This, however, could lead to nitrate-leaching (Seidel et al., 2009). The other scenario was that soil nitrogen content decreases, so the subsequent crop is negatively affected, possibly leading to yield reductions. This was observed in cereal growing, when straw remains at the field: due to its high carbon content, comparable to miscanthus residues remaining on the field, and given that nitrogen content of the soil is low, soil bacteria need the available nitrogen for the turnover of the straw, which reduces the N availability for the subsequent crop (Reinertsen et al., 1984). If one of the two mentioned possibilities for soil nitrogen dynamics applies for a miscanthus removal, both will

lead to inefficiencies: Either the subsequent crop would be negatively affected, leading to lower yields, which would reduce land-use efficiency or nitrogen would be leached, leading to environmental problems (low environmental efficiency) and resulting additionally in a low nitrogen-use efficiency.

As stated above, Lewandowski et al. (2019) emphasised the importance of thinking in whole value chains to achieve a sufficient resource-use efficiency, which includes that biomass quality should be tailored to the needs of its utilization pathway. This is also relevant for miscanthus being used as biogas substrate, for example. Miscanthus biomass quality can be influenced by the choice of either the harvest date or the genotype.

Depending on the harvest date, different lignin and water contents appear, making the biomass more or less suitable for different utilization pathways. Lower lignin contents are preferable for anaerobic digestion and thus miscanthus should be harvested green before winter.

Miscanthus has a high nutrient-use efficiency if it is harvested brown after winter, which is mainly due to its efficient nutrient recycling every year. By harvesting miscanthus 'green' in autumn, however, this nutrient recycling may be impaired, as the crop has less time to relocate its nutrients to the rhizome. This can also negatively affect the crop's lifetime, as it was shown by Fritz & Formowitz (2010): In their study, miscanthus was harvested two following years in August, with the result of decrease in dry matter yield of 30% compared to a brown harvest, which was conducted in spring in two following years. Consequently, miscanthus should be harvested as late as possible in autumn, to increase the nutrient-use efficiency and thus ensure a long lifetime of the crop.

On the other hand, the land-use efficiency of miscanthus rises with increasing amounts of methane produced per hectare agricultural land. Methane hectare yield can be calculated by the dry matter yield and the substrate-specific methane yield. Following the results of Wahid et al. (2015), the methane hectare yield of miscanthus is positively correlated with the dry matter yield. However, Larsen et al. (2014) have shown that a delayed autumn harvest goes ahead with (dry matter) yield reductions of 0.24 - 0.32% per day on average, for example due to leaf losses. In addition to that, a later harvest increases lignin contents in miscanthus biomass, which reduces biodegradability and leads to lower substrate-specific methane yields. Consequently, a later harvest in autumn might not only lead to lower dry matter yields but also to lower substrate-specific methane yields, reducing the methane hectare yield of miscanthus. Hence, to increase land-use efficiency by high methane yields of miscanthus, the crop should be harvested as early as possible in autumn to avoid leaf losses and a lower biodegradability.

However, even if the optimal harvest date for biogas production is found, a further issue has to be dealt with: storing, which is indispensable to ensure biomass supply for a continuous process of anaerobic digestion (Vervaeren et al., 2010). Brown-harvested miscanthus, with its moisture contents lower than 15%, can easily be stored at a covered and ventilated area (Kirschbaum et al., 1995; Lewandowski et al., 2000). In contrast to that, the green-harvested miscanthus for anaerobic digestion has higher moisture contents, and thus ensiling of miscanthus seems to be the method of choice to store the biomass (Baldini et al., 2017).

By ensiling miscanthus, again the question arises, when to harvest it to reach sufficient silage quality. A sufficient silage quality is important, as otherwise the methane hectare yield would be reduced for example due to mould (Szymańska et al., 2014). In addition to that, fermentation acids are produced during ensiling, which probably goes along with energy losses and leads also to a decrease in the methane hectare yield. Both examples lead to losses, which would reduce the land-use efficiency of miscanthus as biogas substrate. Consequently, the chosen harvest date has to ensure not only a high digestibility and long-term productivity of miscanthus, but also a sufficient silage quality to achieve high methane yields and thus a high land-use efficiency.

Following the results of Lewandowski et al. (2016), such miscanthus genotypes have to be chosen, which are optimally suited to the potential end-use of the biomass. This is for example due to different proportions of leaves and stems in miscanthus genotypes, leading to different suitabilities for various utilization pathways. As an example, Baxter et al. (2014) have shown that stems are more suitable for combustion due to their higher calorific value. Wahid et al. (2015), however, demonstrated a higher substrate-specific methane yield of leaf fraction compared to stem fraction. The question is, which genotype should be chosen to achieve high efficiencies, if miscanthus is used as biogas substrate: On the one hand, this genotype should have a high land-use efficiency, which is based on high methane hectare yields. Associated with that, the biomass of the genotype should be suitable for ensiling, leading to a sufficient silage quality without negative impacts on the methane hectare yield. On the other hand, the genotype should tolerate a green cut in autumn, which requires an efficient nutrient-management to ensure the long lifetime and thus the land-use efficiency of the crop.

1.3 Aim of the study

Miscanthus can be seen as a promising bioeconomy crop. To be environmentally benign from a bioeconomic point of view, it should be resource-use efficient from different perspectives. Generally, efficiency is defined as the proportion of a value-based output to a value-based input (Wöhe & Döring, 2010). In the case of this study, it means that miscanthus achieves a high land-use, nutrient-use and environmental efficiency. As a consequence, the cultivation of miscanthus should be considered as a whole value chain in which single process steps are harmonized with each other.

In the previous chapter, the current inefficiencies in miscanthus cultivation were pointed out. It was illustrated that there exist several propagation methods for miscanthus. Seed propagation is the most promising one, due to its high multiplication rate. However, it is only applicable for fertile hybrids. Thus, rhizome propagation is still the most common propagation way, as it is vegetative and can be applied for each miscanthus genotype. However, this propagation method does not only have a low multiplication efficiency (1:10; Xue et al., 2015) but also a low land-use efficiency, as the mother field is destroyed by harvesting rhizomes.

After the removal of miscanthus a high yield of the subsequent crop is desirable, to achieve also a high land-use efficiency. To reach that, the crop should be highly competitive against resprouting miscanthus to achieve high yields. Additionally, the topic about nitrogen dynamics in soil after a miscanthus removal becomes relevant: Nitrogen should not be leached, because this would lead to environmental problems (low environmental efficiency) and result in a low nitrogen-use efficiency. On the other hand, a deficiency in plant available nitrogen could lead to yield losses of the subsequent crop and thus reduce land-use efficiency.

Finding the optimal harvest date for miscanthus, if it is utilized as biogas substrate, leads to a trade-off in achieving different efficiency aims: If it is harvested early in autumn, a high land-use efficiency could be achieved by gaining high methane yields. However, this probably impairs the nitrogen-use efficiency, leading to a lower lifetime of the crop, which in turn reduces the land-use efficiency. Additionally, the optimal harvest date must lead to a sufficient silage quality to achieve high methane yields and thus a high land-use efficiency. A harvest date including all these issues has to be found, to make the utilization of miscanthus for biogas production most efficient.

Based on the decision, how miscanthus is going to be used, also the miscanthus genotype should be chosen carefully. To reach a high land-use efficiency, high dry matter and substrate-specific methane yields should be achieved, if it is used as biogas substrate. This necessitates that the genotype ensiles well to gain a sufficient silage quality, which in turn leads to high methane hectare yields. Additionally, the chosen genotype should have a high nutrient-use efficiency, which ensures a long productive lifetime and thus a high land-use efficiency of miscanthus.

To summarize, high demands are made on miscanthus to make it efficient from a bioeconomic view. Therefore, the aim of this study was to develop approaches for improving the efficiency of miscanthus biomass production along its supply chain. Referring to the above elaborated research gaps, following research questions were investigated in this study:

I. Establishment

1. How can the efficiency of miscanthus propagation be improved beyond existing propagation methods?

II. Re-integration into crop rotations

1. How can the land-use efficiency after a miscanthus removal be increased?
2. How can the nutrient-use efficiency after a miscanthus removal be optimized?

III. Utilization pathway anaerobic digestion

1. What is the optimal harvest date for miscanthus to achieve a high land- and nutrient-use efficiency?
2. What are optimal genotype characteristics for miscanthus as biogas substrate to achieve a high land- and nutrient-use efficiency?

1.4 Publications

To answer the research questions, several field trials and laboratory experiments were conducted. The results of these investigations were published in four scientific papers, which are included in **Chapter 2** to **4** in the current study.

Thereby, **Chapter 2**, which addresses research question 1, is entitled *"Novel establishment way for miscanthus"* and includes the following publication:

Mangold, A., Lewandowski, I., Xue, S., & Kiesel, A. (2018). 'Collar propagation' as an alternative propagation method for rhizomatous miscanthus. *GCB Bioenergy*, **10**, 186-198. Doi: 10.1111/gcbb.12480.

For the experiments of this publication, plant material from the genotypes *M. x giganteus* (*Mxg*), *M. sacchariflorus* (*MSac*) and *M. sinensis* (*MSin*) was harvested in a non-destructive way and assigned to three propagation categories: collar fragments, collar fragments with additional rhizome pieces and rhizome cuttings as control. In a chamber study, the shoot emergence of the three fragments was analysed. In two field experiments, the performance of the cuttings, which were either transplanted from greenhouse or directly planted into the field, was examined. Additionally, the effect of different harvest dates of collars on shoot emergence has been examined in a further chamber study.

Chapter 3 is entitled *"Reintegration of miscanthus fields into a crop rotation"* and includes the following publication:

Mangold, A., Lewandowski, I. & Kiesel, A. (2019). How can miscanthus fields be reintegrated into a crop rotation? *GCB Bioenergy*, **11**, 1348-1360. Doi: 10.1111/gcbb.12636.

To answer research question 2, four different spring crops (ryegrass, rapeseed, barley, maize) and as control fallow were cultivated after a mechanical miscanthus removal in spring 2017. Referring to research question 3, the plots of each subsequent crop (except fallow land) were divided into 'fertilized' and 'unfertilized' crops. The resprouting of miscanthus in the subsequent crops and fallow land as well as soil-nitrogen content (NO_3-) were analysed. After the subsequent crops were harvested, winter wheat was sown and treated under same conditions over the whole trial to finally determine resprouting of miscanthus.

Chapter 4, entitled "*Miscanthus for biogas production: The effect of genotype, harvest date and ensiling on digestibility and methane hectare yield of miscanthus*", includes two publications:

4.1 Mangold, A., Lewandowski, I., Möhring, J., Clifton-Brown, J., Krzyżak, J., Mos, M., Pogrzeba, M., Kiesel, A. (2019). Harvest date and leaf:stem ratio determine methane hectare yield of miscanthus biomass. *GCB Bioenergy*, **11,** 21-33. Doi: 10.1111/gcbb.12549.

4.2 Mangold, A., Lewandowski, I., Hartung, J., & Kiesel, A. (2019). Miscanthus for biogas production: Influence of harvest date and ensiling on digestibility and methane hectare yield. *GCB Bioenergy*, **11,** 50–62. Doi: 10.1111/gcbb.12584.

To find answers on research question 4, a field trial was established, on which miscanthus was harvested over two years (2016 and 2017) on three different harvest dates, starting in mid-September, beginning of October and mid-October. To answer research question 5, the field trial included four different miscanthus genotypes, namely *Mxg*, *GNT1*, *GNT3* and *Sin55*. At each harvest date, dry matter yield (DMY) and dry matter content (DMC) of leaf and stem for every genotype were determined. Additionally, biogas batch tests were conducted to measure substrate-specific methane yield (SMY) of leaf and stem. The results were used to calculate leaf and stem methane hectare yield (MY). Furthermore, in both years cellulose, hemicellulose, lignin and ash content of both, leaf and stem, were analysed. In addition to that, nitrogen (N), potassium (K) and phosphorus (P) content of leaf and stem were investigated in 2016 and 2017. The outcomes of these analyses resulted in **Chapter 4.1**.

Additionally, to answer research questions 4 and 5, a second study was conducted, in which the harvested biomass of the four genotypes was used for an ensiling trial. In the year 2017, at each of the mentioned harvest dates (mid-September, beginning of October and mid-October) a part of the harvested miscanthus biomass was ensiled in WECK®- glasses and stored for 90 days. After those 90 days, the ensiled biomass was analysed for its silage quality. Additionally, a biogas batch test was conducted, which included ensiled and non-ensiled biomass, to analyse the effect of ensiling on the SMY and MY. The outcomes of these analyses are presented in **Chapter 4.2**.

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2 Novel establishment way for miscanthus

In this chapter, a novel propagation method for miscanthus via *collars* was tested. For this purpose, several field and chamber studies were conducted in which collar fragments were compared with two other propagation fragments of three different miscanthus genotypes.

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'Collar propagation' as an alternative propagation method for rhizomatous miscanthus

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Abstract

The demand for perennial nonfood crops, such as miscanthus, is increasing steadily, as fossil resources are replaced by biomass. However, as the establishment of miscanthus is very expensive, its cultivation area in Europe is still small. The most common propagation method for miscanthus is via rhizomes, the harvesting of which is very labour-intensive. Seed propagation is promising, but not suitable for sterile genotypes. In this study, a new vegetative propagation method, 'collar propagation', was tested in field and controlled environment studies. Collars are built at the junction between rhizome and stem. They can be harvested in a less destructive way than rhizomes by pulling out the stems from winter-dormant miscanthus plants. One genotype of each of the species *M. sacchariflorus*, *M.* × *giganteus*, *M. sinensis* in combination with three fragment types (collars, rhizomes, collars + rhizomes) were tested for establishment success and plant performance. The performance (e.g. dry matter yield) of collar-propagated plants was either better than or not significantly different from rhizome-propagated plants. Pregrown plantlets transplanted into the field showed no significant differences in establishment success between the fragments within a genotype. When directly planted into the field however, the fragment 'rhizome+collar' had a significantly better establishment success than the other two. The winter survival rate of the fragment 'rhizome+collar' was 70% for *M. sacchariflorus* and 75% for *M.* × *giganteus*. Emergence success from collar-derived plants was not affected by harvest date (harvested monthly from November to February). This study showed that miscanthus propagation via collars is feasible and a promising alternative to rhizome propagation, as the multiplication rate of collars is comparable to that of rhizome propagation. Collar propagation is the more suitable method for the tested genotypes of the species *M. sacchariflorus* and *M.* × *giganteus*, but not for *M. sinensis* genotypes, which may be better propagated by seeds.

Keywords: collar, establishment, miscanthus, overwintering, propagation, rhizome

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Introduction

As more and more fossil resources are being replaced by biomass for various energetic and material utilization options, the demand for biomass is increasing steadily. To satisfy the goals of a growing bioeconomy, this biomass needs to be produced sustainably and conflicts between food security and bioenergy avoided. For this purpose, perennial nonfood crops, such as miscanthus, offer a viable option thanks to their generally low-input requirements and high yield potential, also under conditions marginal for the production of food crops (McCalmont *et al*., 2017).

Miscanthus is a perennial rhizomatous C_4 grass originating from South-East Asia. Typical yields of the most commonly grown, and so far only commercially available, genotype *Miscanthus* × *giganteus* range between 15 and 25 Mg dry matter ha^{-1} yr^{-1} in temperate climates (Lewandowski *et al*., 2000; Heaton *et al*., 2004; Lesur *et al*., 2013; Boersma & Heaton, 2014a; Iqbal *et al*., 2015). The low-input character of miscanthus can be mainly attributed to its perennial nature, with a lifetime of more than 20 years and efficient nutrient recycling (Cadoux *et al*., 2012). Given its high yield potential and benign environmental profile, miscanthus is seen as a promising crop to provide sustainably produced biomass for a growing bioeconomy (Lewandowski, 2015).

Despite these advantages, miscanthus is currently only grown on about 19 000 ha in Europe (Lewandowski *et al*., 2016). Reasons for this are a lack of higher value utilization options and high initial investment costs for establishment of the plantation. Novel higher value utilization options have only recently been identified and need to be implemented in practice to create a market for miscanthus biomass (Kiesel & Lewandowski, 2017; Lewandowski *et al*., 2016; van der Weijde *et al*., 2017). However, expensive propagation is still one of the main reasons for the low cultivation rate. Therefore, various studies have tested alternative propagation

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methods (Lewandowski *et al*., 2003; Atkinson, 2009; Zub & Brancourt-Hulmel, 2010; Xue *et al*., 2015). The conventional propagation method for miscanthus is via rhizomes. This is currently the cheapest and easiest of all available propagation methods. However, a rhizome harvest is not possible every year because harvesting all rhizomes leads to the destruction of the complete mother field and leaving some rhizomes in the field to regrow the following year requires time for the plants to recover. The low dividing efficiency of 1:10–50 is a further disadvantage of rhizome propagation (Xue *et al*., 2015; Clifton-Brown *et al*., 2017). Heaton *et al*. (2010) showed that rhizomes harvested from 0.4 ha can result in about 3.6 ha of miscanthus, and a such vegetative reproduction is still economically viable. To improve the multiplication rate and reduce costs, other vegetative propagation methods have been sought. One alternative propagation method is micropropagation, which is very effective due to its high multiplication rate (1:960) and has the additional benefit of being able to prevent the transmission of diseases (Lewandowski, 1998; Xue *et al*., 2015). This method is the most expensive way of propagating miscanthus, because it is very labour-intensive (Xue *et al*., 2015), and is therefore mainly used for scientific trials. Propagation via seeds is another promising method and with the development of novel hybrids that produce fertile seeds, this method is becoming increasingly interesting and relevant. It has a much higher multiplication rate than rhizome propagation (Clifton-Brown *et al*., 2017) and enables long use of the mother plants, as seeds can be harvested without destroying the propagation fields. For this reason, seed propagation seems a viable method for fertile hybrids. However, propagation via seeds increases the danger of invasiveness, an important factor when selecting plants to be used as new biomass crops (Raghu *et al*., 2006; Boersma & Heaton, 2014b). Thus, future genotypes of miscanthus may be sterile, rendering seed production impossible. For such genotypes, an improved vegetative, nondestructive and low-cost propagation method is required.

Collar propagation is a vegetative propagation method, which could be used for sterile genotypes. Collars are built at the junction between the rhizome and the stem (Fig. 1) and usually have buds. Theoretically, every bud has the potential to generate a new shoot or even a new plant (Klimešová & Klimeš, 2007). The collars can be harvested less destructively than rhizomes by pulling out the stems of senesced plants, leaving the rhizomes in the ground. As the collars are strongly attached to the bottom of the stem, the chance is quite high that stem and collar can be harvested in this way. As enough of the rhizome is left in the ground, the propagation field is not destroyed. This ensures long-term use of the field, avoiding the establishment of new propagation fields.

So far, there have been no reports on the potential of using this propagation method for miscanthus. Hence, the objective of this study was to test whether it is possible to raise new miscanthus plants from collar fragments. For this purpose, three different fragment types (rhizomes, collars with additional rhizome pieces and collars only, see Fig. 1) from one genotype of each of the species *M. sacchariflorus*, *M.* × *giganteus* and *M. sinensis* were tested in three trials. Establishment success, yield and plant performance were analysed. Additionally, the best harvest date for collars in terms of establishment success was investigated.

Materials and methods

The plants used as material source were taken from plots of the field trials established in the European Miscanthus Improvement (EMI) project (Clifton-Brown *et al*., 2001) in 1997 at the experimental station Ihinger Hof (IHO, 48°45′N, 8°56′E, 480 m a.s.l.). Of the 15 genotypes tested in this trial, three were chosen (EMI numbers 4, 5 and 11) for this study to give a genotypic diversity: one genotype from each of the species *M.* × *giganteus (M×G)*, *M. sacchariflorus (MSac)* and *M. sinensis (MSin)*, as shown in Table 1 (in accordance with Clifton-Brown *et al*., 2001). In late April 2014, the stubble and rhizomes were harvested in a nondestructive way by pulling the whole stems with attached collar and rhizome parts out. They were washed,

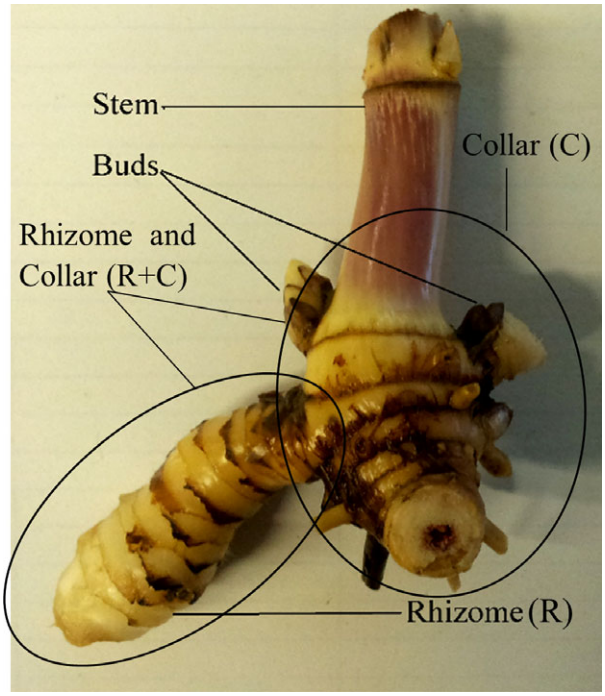

Fig. 1 Photograph of a *Miscanthus sacchariflorus* Rhizome + Collar fragment used in the experiments.

cut and separated into three treatment categories according to fragment size: 5-cm-long collar fragments (C), 5-cm-long collar fragments with additional 3-cm-long rhizome pieces (R+C) and 5-cm-long rhizome cuttings (R) as a control. The 5-cm collar fragments were cut in such a way that they were made up of 2 cm aboveground stem and 3 cm belowground collar part. All these prepared materials were then placed in plastic bags and stored in the fridge at 4 °C until they could be used in the experiments. The maximum storage period was 3 weeks. Four different experiments were conducted to analyse the viability of the collar fragments, with genotype and fragment size as treatment factors. Table 1 shows all material types for all genotypes.

An overview of the different trials is provided in Table 2, including the genotypes and fragment types used and the parameters tested. The different treatments are combinations of genotype (*M×G*, *MSac* or *MSin*) and fragment type (C, R+C or R) and are thus abbreviated, for example, to '*MSac* R+C' for the fragment collar + rhizome of the genotype *Miscanthus sacchariflorus*.

Shoot emergence from fragments in a chamber study

The first experiment (Trial 2.1) started in mid-May 2014 when the propagules had been stored for 20 days. The fragments were planted in 54 pots measuring 18 cm (length) × 12 cm (width) × 6 cm (height) each, which were filled with 550 g soaked potting media (100% water-holding capacity) with a high clay content of 10–12%, a pH value of 5.5–6, and containing all necessary micronutrients (Ensinger Kulturerden, Pikiersubstrat Premium). For each treatment combination, six randomly selected fragments were planted in each pot and covered with an additional 150 g soaked potting media (1 cm minimum soil coverage). In addition, each pot was covered with a thin transparent film to avoid water loss. On the fifth day after planting, the film was removed and, from this point onwards, the pots were watered with 50 ml water a day. The pots were placed in a randomized complete block design in a climate chamber with a 16 h/8 h light/dark period and a 23 °C/18 °C day/night temperature for a period of 18 days, creating optimal conditions for establishment. During this time, the sprouting of new shoots was recorded on a daily basis, counting shoots that had emerged from soil by at least 1 cm.

Field performance of transplanted chamber study plantlets

At the end of the chamber study in June 2014, the strongest plantlets of each genotype from Trial 2.1 were taken and manually transplanted into a clayey loam research field at the University of Hohenheim campus (48°42′N, 9°13′E) (Trial 2.2). To ensure good soil conditions, the field was harrowed before planting. The three different fragment types of two genotypes (*M×G* and *MSac*) were transplanted in a randomized complete block design with three replicates, that is a total of 18 plots. Due to the low emergence of *MSin* in the chamber study, this genotype was neglected in this trial. In each of the 18 plots (1.0 m × 0.3 m), four plantlets of either *M×G* and *MSac* were

Table 1 Description of the different miscanthus genotypes with their EMI numbers and their fragment weights (n = 3)

		Fresh weight (g)/fragment		
Genotype	EMI No.	5-cm collar (C)	5-cm collar with 3-cm rhizome (R+C)	5-cm rhizome (R)
Miscanthus sacchariflorus (*MSac*)	5	4.4 ± 1.4^{b}	8.3 ± 1.3^{a}	4.5 ± 0.7^{b}
Miscanthus × *giganteus* (*M×G*)	4	5.6 ± 1.8^{B}	7.7 ± 1.3^{A}	4.3 ± 0.7^{C}
Miscanthus sinensis (*MSin*)	11	3.0 ± 0.9$^{\boldsymbol{b}}$	4.7 ± 1.1$^{\boldsymbol{a}}$	2.9 ± 0.7$^{\boldsymbol{b}}$

Further information on these genotypes is available in the references of Clifton-Brown *et al.* (2001) and Iqbal & Lewandowski (2014). Significant differences of weight within a genotype are indicated by different lower-case letters (a, b) for *MSac*, different upper-case letters for *MxG* (A, B, C) and different bold, italic letters (***a***, ***b***) for *MSin* (α = 0.05).

Table 2 Overview of the four trials

Trial	Abbreviation	Trial type	Genotypes	Fragments	Measured traits	Date range
2.1 Shoot emergence in a climate chamber	Chamber study	Chamber study	*MSin* *M×G* *MSac*	C; R+C; R	Shoot emergence	19/05/2014 to 06/06/2014
2.2 Field performance of transplanted greenhouse plantlets	Transplanted	Field trial	*M×G* *MSac*	C; R+C; R	Plant traits Establishment success	06/06/2014 to mid-April 2015
2.3 Field performance of fragments directly planted into the field	Directly planted	Field trial	*MSin* *M×G* *MSac*	C; R+C; R	Plant traits Establishment success	06/06/2014 to mid-April 2015
2.4 Influence of collar harvest date on emergence	Harvest trial	Chamber study	*M×G* *MSac*	C	Shoot emergence	Early November 2014 to early February 2015

planted with 0.2 m spacing between plants within the rows. The plots were irrigated twice in the first 2 weeks after planting, and then, no additional water or fertilizer was given during the whole experimental period. Weeding was conducted several times in all plots to ensure better establishment conditions. The establishment success was determined in July 2014.

At the end of the 2014 growing season (3rd November 2014), plant survival was assessed prior to harvesting. After that, the three strongest plants per plot were selected for morphological measurements including plant height, stem number and stem diameter. Plant height was measured from the soil surface to the node of the uppermost fully expanded leaf on the highest stem of each selected plant. Stem diameter was measured on the same stem between the collar and the first internode. For stem number per plant, all stems with a height of at least 10 cm were counted and the number divided by the planting density. The harvested plants were then oven-dried (60 °C for 7 days) and weighed for dry matter biomass yield assessment. To calculate overwintering survival rate, the plants still alive after the winter (mid-April 2015) were counted.

Field performance of fragments directly planted into the field

A second field experiment started at the same time as Trial 2.2, but this time planting the stored fragments directly into the field (Trial 2.3). In each 1.2 m × 0.6 m plot, one fragment type of one genotype was planted. The trial had a randomized block design with four replications, giving 36 plots in total. Per plot, 10 fragments were planted at a soil depth of 5 cm. Prior to planting, the field was harrowed to ensure good establishment conditions. The plots were irrigated twice in the first 2 weeks after planting, and then, no additional water or fertilizer was given during the whole experimental period. To minimize weed pressure, manual weeding was conducted several times in all plots. In July 2014, 1 month after planting, the establishment success was calculated as a percentage of the planted fragments.

Morphological measurements were taken of the three strongest plants at the end of the 2014 growing season. Stem number, stem diameter, plant height, biomass yield and number of plants at harvest were determined according to the methods described for Trial 2.2. In April 2015, overwintering survival was assessed.

Influence of harvest date on shoot emergence from collars

During the period November 2014 to February 2015, collar pieces of *M*×*G* and *MSac* were collected each month from the EMI project fields at Ihinger Hof (Trial 2.4). A separate shoot emergence experiment was conducted for each harvest date. Thirty collar fragments were randomly selected from each genotype and planted into five pots with six collars each, in the same way as in the chamber study (Trial 2.1). The emergence ratio of collar pieces from each harvest date was calculated 21 days after planting.

Statistical analysis

Data analysis was performed using the Statistical Analysis Software SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA). Metric plant traits were analysed by mixed models using the PROC MIXED procedure. A test for normal distribution and variance homogeneity was conducted for each plant trait. As emergence rate and establishment success in each trial are binomially distributed, a generalized linear mixed model was performed using the PROC GLIMMIX procedure and a logarithm was used for the link function. The model allows overdispersion. In Trials 2.1–2.3, establishment success was measured by shoot emergence, and where appropriate, survival rate after transplanting, at harvest and after winter, was analysed using the generalized linear mixed model shown in Eqn (1). In the Trials 2.2 and 2.3, plant traits were analysed by the linear mixed model shown in Eqn (2). In both models, genotype, fragment and their interactions were taken as fixed effects. In Trials 2.1–2.4, the effects of replicates were assumed to be random effects. In Trial 2.4, Eqn (3) was used to analyse shoot emergence. This model is the same as Eqn (1) but with fragment instead of harvest date. In this trial, shoot emergence of collars from different harvest dates was measured in different experimental runs, and thus, the estimated error only accounts for errors within the chamber experiment and ignores errors in the different experimental runs. As such, it underestimates the true error variance. Multiple *t*-tests with a significance level of $\alpha = 0.05$ were conducted only where significance was found in a type 3 test for fixed effects. The genotype *MSin* was partially omitted from the statistical analysis and presentation of results due to its poor emergence.

$$y_{ijk} = \mu + \log\,(g_i + f_j + (gf)_{ij} + r_k) + e_{ijk} \quad (1)$$

$$y_{ijk} = \mu + g_i + f_j + (gf)_{ij} + r_k + e_{ijk} \quad (2)$$

$$y_{ihk} = \mu + \log\,(g_i + d_h + (gd)_{ih} + r_k) + e_{ihk} \quad (3)$$

y_{ijk} = measurement of the *g*-th genotype with the *f*-th fragment in *r*-th replication; μ = general effect; g_i = main effect of the *g*-th genotype (*MSac*; *M*×*G*; *MSin*); f_j = main effect of *f*-th fragment (C, C+R, R); d_h = main effect of *h*-th harvest date (November, December, January, February); bc_{gf} = interaction of *g*-th genotype with *f*-th fragment; r_k = random effect of *r*-th replication; e_{ijk} = residual error term for y_{ijk}; e_{ihk} = residual error term for y_{ihk}.

Results

Establishment success

Overall, the trials showed that the establishment success of the collars was either better than or at least as good as that of the rhizome fragments. To gain an overview of the development of fixed effects over the 18 days of the chamber study (Trial 2.1), days 6, 12 and 18 were taken for analysis. The fixed effects showed that genotype and fragment were significant, whereas the

interaction between genotype and fragment was not significant on these days (Table 3). It was found that the rhizome fragments of all three genotypes had the lowest emergence rate on all 3 days (except *MSac* on day 6; Fig. 2). The fragment 'R+C' had the highest emergence rate at each of the days 6, 12 and 18 (except for *MSin* R+C at day 6). The fragment 'C' was in between. At least 54% of the collar fragments of all three genotypes had emerged after 18 days under controlled conditions. Information on shoot emergence of each day can be found in Table S1.

When the pregrown plantlets were transplanted into the field (Trial 2.2), there was no significant difference in survival rate between the fragment types (Fig. 3a). When planted directly into the field (Trial 2.3), at least 40% of the collar fragments of genotypes *M*×*G* and *MSac* emerged (Fig. 3b). Fragment R+C had the highest emergence rates in all three genotypes. The rhizome fragments had lowest emergence rate, except for *MSin*, where the collars had the lowest emergence, but without a significant difference. For *MSac*, the fragment R+C had significantly higher emergence rates than the rhizomes. For *M*×*G*, the R+C fragment had significantly higher emergence rates than both other fragments (Fig. 3b).

Field establishment success was determined twice, at harvest in November 2014 (Fig. 4a) and after the winter in April 2015 (Fig. 4b). On both dates, survival of the transplanted plants (Trial 2.2) was higher than that of the directly planted fragments (Trial 2.3). The directly planted collar fragments showed a similar or significantly higher survival rate than the rhizome fragments, whereas none of the *MSin* collar fragments had survived (Fig. 4a,b) on either assessment date. For the transplanted plants, no significant effect was found between the different fragment types within the two genotypes tested. Although there were some losses (about 5%) from counted plants between November 2014 and April 2015 for *MSac* C, the general losses of Trial 2.2 over winter were low. In Trial 2.3, however, the overwintering losses for *MSac* *R*+C, *M*×*G* C and *M*×*G* R were between 10 and 15%, and thus higher than for Trial 2.2 for these combinations. It should be pointed out that in the case of *MSac* C (directly planted), more plants were counted in November 2014 and after winter in April 2015 than at the first counting in July 2014. There could be two reasons for this: First, the planting distance was narrow at only 20 cm. Therefore, it was difficult to differentiate between plants and their tillers

Table 3 Type 3 test for the significance of main effects and their interactions (genotype, fragment, genotype*fragment) on shoot emergence of the three genotypes (*MSac*, *MxG* and *MSin*) in the chamber study (Trial 2.1). Level of significance was α = 0.05

	Day 6		Day 12		Day 18	
	F-value	Pr>*F*	*F*-value	Pr>*F*	*F*-value	Pr>*F*
Genotype	6.67	0.0032	5.54	0.0076	8.93	0.0006
Fragment	5.82	0.0061	14.86	<0.0001	13.80	<0.0001
Genotype × Fragment	2.5	0.0582	2.34	0.0724	1.68	0.1740

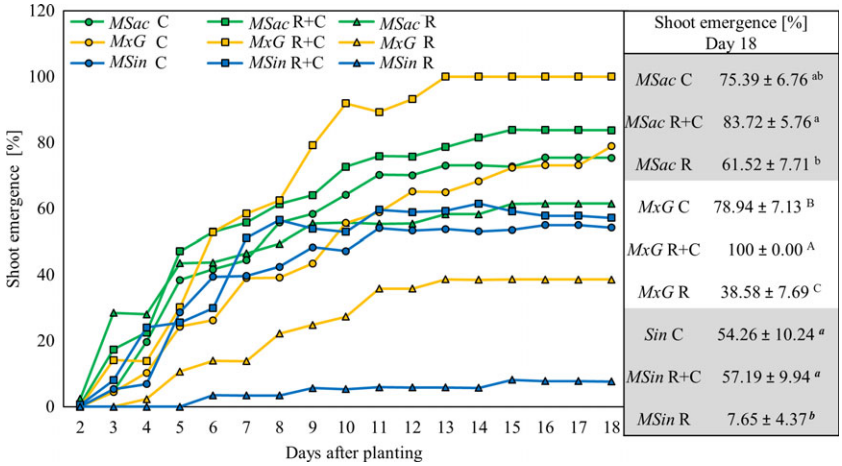

Fig. 2 Shoot emergence (*n* = 6) over 18 days after planting in a chamber study (Trial 2.1) with the three genotypes (*MSac*, *M*×*G* and *MSin*) and the three fragment types (collar = C, rhizome and collar = R+C, rhizome = R). Significant differences within each genotype are indicated by different lower-case letters for *MSac*, upper-case letters for *M*×*G* and bold italic letters for *MSin* (α = 0.05).

and double counting may have occurred. In addition, it is possible that some plants emerged later than July 2014 and therefore were not included in the first counting.

Influence of fragment type on plant performance

This section shows the results of Trials 2.2 and 2.3, referred to as 'Transplanted' and 'Directly planted', respectively.

The type 3 test (Table 4) showed that overall only very few significant impacts were observed for the main effects genotype and fragment. The interaction of genotype and fragment only showed a significance for stem number. Fragment only showed significant effects in Trial 2.3 for the traits dry matter content and height. The trait stem diameter was not significantly influenced by the main effects or their interaction, and for this reason, these results are not shown in detail below. Where the pregrown plantlets were transplanted into the field, R+C fragments resulted in the shortest and R fragments in the tallest plants, for both genotypes (Fig. 5a). However, the differences were not significant. Where the fragments were directly planted into the field, the results were the reverse: R+C fragments had in the tallest, and R fragments the shortest plants, for both genotypes (Fig. 5b). However, this difference was only significant for *M*×*G*.

In Trial 2.2, no significant differences were detected between the different fragments for *MSac* (Fig. 5a). For *M*×*G*, however, there was a significant difference between the fragments R+C and R. There was a difference in stem number between genotypes *MSac* and *M*×*G* for the fragment R. It was highest in *M*×*G* and lowest in *MSac*. In Trial 2.3, for *MSac*, fragment C had a significantly higher stem number than R, with R+C in between the two (Fig 5b). For *M*×*G*, the same trend was observed, but the differences were too small to be significant.

The stem diameter of plants from the three fragments was not significantly different within each genotype in either trial. On average, *MSac* and *M*×*G* showed a stem diameter of 0.68 cm and 0.79 cm in Trial 2.2 and

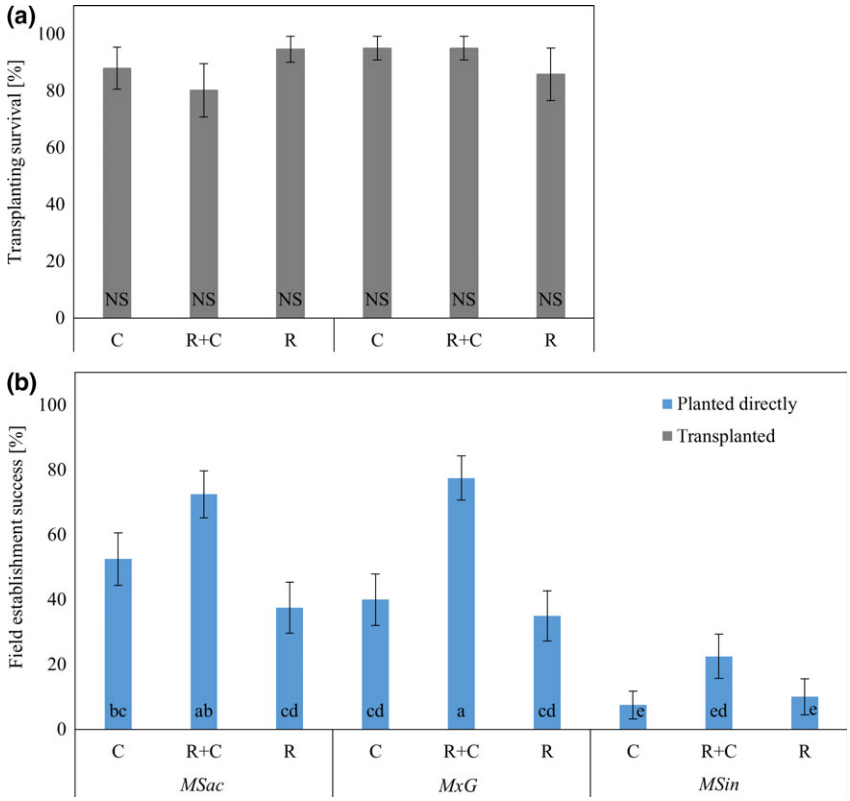

Fig. 3 Transplanting survival of pregrown plantlets (a) and field establishment success of fragments planted directly into the field (b) one month after transplanting/planting. Transplanting survival was assessed for two genotypes (*MSac* and *M*×*G*) and three fragment types (collar = C, rhizome and collar = R+C, rhizome = R). Field establishment was conducted for all three genotypes (*MSac*, *M*×*G* and *MSin*) in combination with the three fragment types. Significant differences between the genotypes in combination with the fragments are indicated by different upper-case letters for transplanted pregrown plantlets and different lower-case letters for fragments planted directly into the field ($\alpha = 0.05$). Bars represent standard deviation.

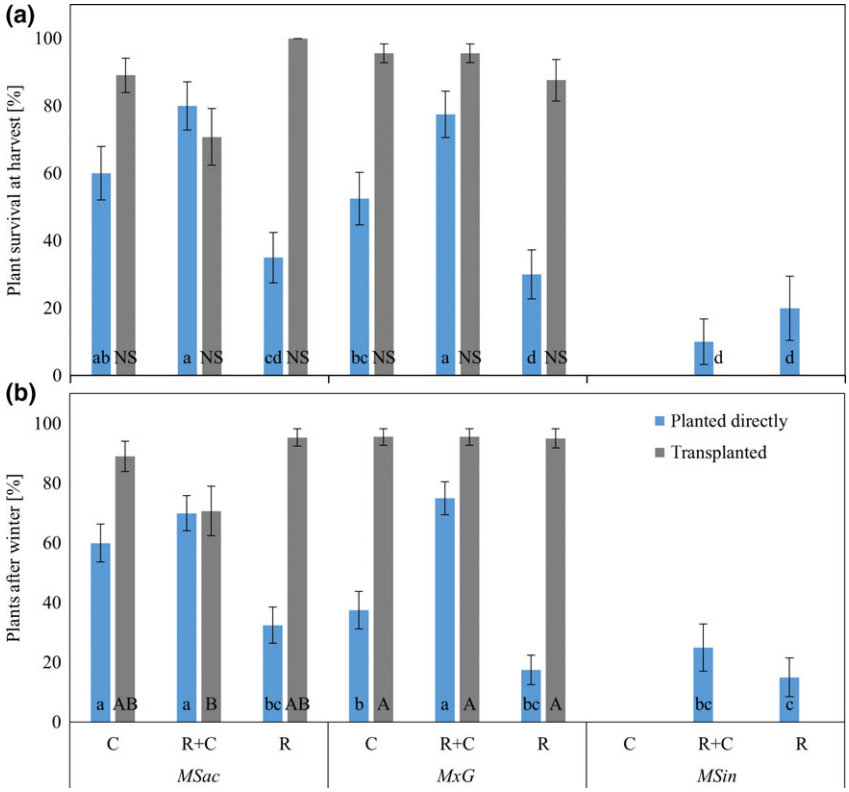

Fig. 4 Plant survival [%] at harvest in November 2014 (a); and after winter in mid-April 2015 (b); for the three genotypes (*MSac*, *M*×*G* and *MSin*) and the three fragment types (collar = C, rhizome and collar = R+C, rhizome = R) for transplanted pregrown plantlets (Trial 2.2) and fragments planted directly into the field (Trial 2.3). In Trial 2.2, only two genotypes (*MSac* and *M*×*G*) were transplanted. Significant differences between the genotypes in combination with the fragments are indicated by different upper-case letters for transplanted pregrown plantlets and different lower-case letters for fragments planted directly into the field ($\alpha = 0.05$). Bars represent standard deviation.

Table 4 Type 3 test for the significance of the main effects and their interactions (genotype, fragment, genotype*fragment) on yield and plant traits for two genotypes (*MSac* and *MxG*) in two field trials (transplanted, directly planted) ($\alpha = 0.05$).

		Transplanted		Directly planted	
Trait	Effect	*F*-value	Pr>*F*	*F*-value	Pr>*F*
Dry matter yield	Genotype	7.87	0.0205	3.43	0.0838
	Fragment	2.64	0.1252	0.09	0.9158
	Genotype × Fragment	1.05	0.3877	0.22	0.8077
Dry matter content	Genotype	0.23	0.6396	2.03	0.1750
	Fragment	1.66	0.2432	5.48	0.0163
	Genotype × Fragment	0.06	0.9434	0.40	0.6772
Stem number	Genotype	0.29	0.6026	4.84	0.0439
	Fragment	0.55	0.5937	3.37	0.0617
	Genotype × Fragment	5.01	0.0345	1.61	0.2333
Stem diameter	Genotype	2.61	0.1409	2.04	0.1738
	Fragment	0.29	0.7584	0.61	0.5552
	Genotype × Fragment	0.16	0.8553	0.01	0.9911
Height	Genotype	7.42	0.0234	37.39	<0.0001
	Fragment	4.03	0.0562	5.04	0.0212
	Genotype × Fragment	0.11	0.8987	1.01	0.3869

0.65 cm and 0.73 cm in Trial 2.3, respectively. In Trial 2.2, the largest stem diameter was found in plants of *M*×*G* C (0.83 cm) and the smallest in *MSac* R+C (0.64 cm). In Trial 2.3, *M*×*G* C (0.76 cm) also had the largest stem diameter and *MSac* R (0.61 cm) had the smallest.

Dry matter yield (DMY) of the plants from the directly planted fragments (Trial 2.3) was not significantly different within each genotype (Fig. 6b). In Trial 2.2, significant differences were observed between the different fragments of genotype *MSac*. *MSac* C had the significantly highest DMY (36.17 g plant^{-1}) and *MSac* R+C the lowest (26.41 g plant^{-1}). For *M*×*G*, no significant differences were visible; fragment R had the highest yield (25.27 g plant^{-1}; Fig. 6a). For the transplanted plants, the DMC ranged from 41.7% to 47.6% (Fig. 6a). Fragment R+C had highest DMC for both genotypes. Genotype *MSac* showed no significant differences in DMC between the fragments. *M*×*G* R+C, however, had a significantly higher DMC than *M*×*G* R. When planted directly, the DMC varied from 34.9% to 52.7%. Fragment R showed the highest DMC in both genotypes, but without significant differences (Fig. 6b).

Influence of harvest date of collars on emergence ratio

The effect of harvest date of the collar fragments on the emergence rate was not significant at a significance level of $\alpha = 0.05$, whereas the genotype effect was (Table 5). However, the November harvest resulted in the lowest emergence rate in both genotypes: 66.9% for *MSac* and 70% for *M*×*G* (Table 6). On the other harvest dates, the *M*×*G* collars in particular showed very high emergence rates of on average 91.2%. By contrast, for *MSac*, the highest emergence rate of 83.4% was observed for collars harvested in December.

Discussion

This study showed that the tested miscanthus genotypes of the two species *MSac* and *M*×*G* can be successfully propagated and established via collar fragments. These collars can be harvested by pulling out the stems of senesced plants with low impact compared to the current commercial practice of rhizome harvesting. However, the tested genotype of the species *MSin* showed that collar propagation is not suitable for all miscanthus genotypes. The following sections discuss (i) the suitability of the three lower stem parts for

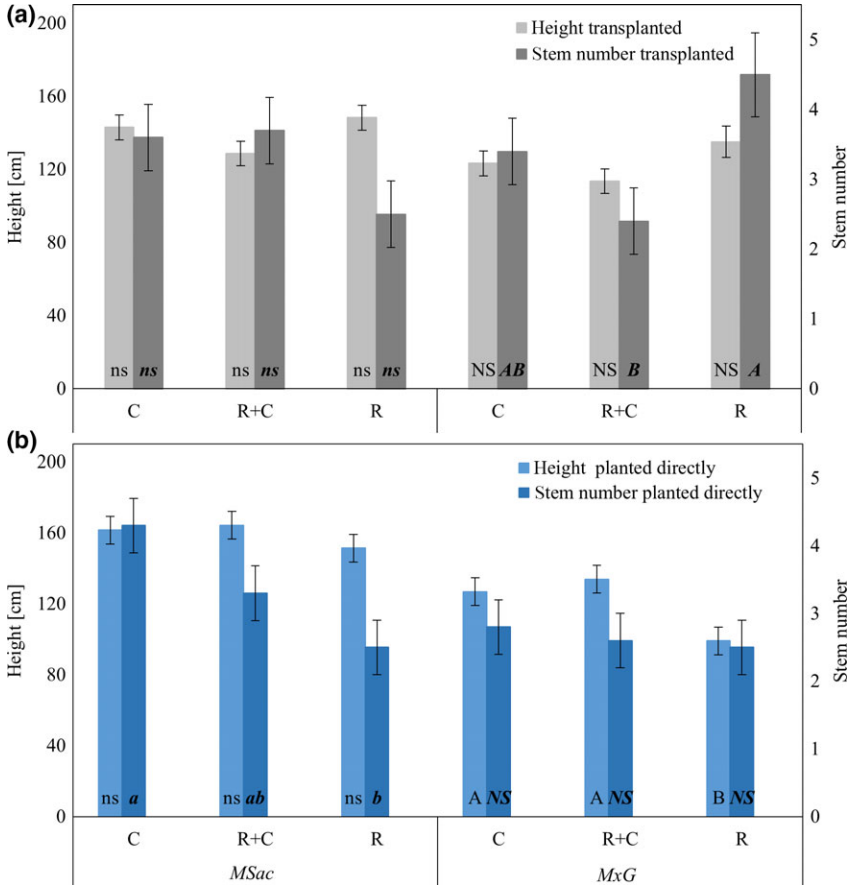

Fig. 5 Height (cm) and stem number of the two genotypes (*MSac* and *M*×*G*) for transplanted pregrown plantlets (a) and fragments planted directly into the field (b). Significant differences in height are indicated by different lower-case letters for *MSac* and different upper-case letters for *M*×*G*. Significant differences in stem number are indicated by bold italic letters, lower-case for *MSac* and upper-case for *M*×*G*. Level of significance was $\alpha = 0.05$. Error bars represent standard deviation in height and stem number.

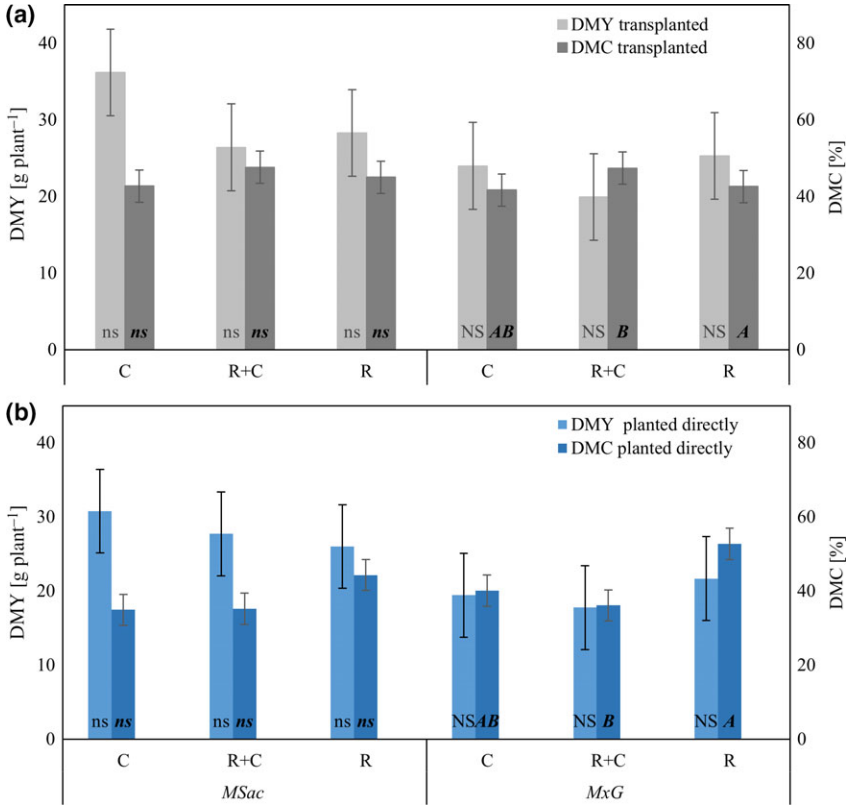

Fig. 6 Average dry matter yield (DMY) [g plant^{-1}] and dry matter content (DMC) [%] of the two genotypes (*MSac* and *M*×*G*) for transplanted pregrown plantlets (a) and fragments planted directly into the field (b). Significant differences in DMY are indicated by different lower-case letters for *MSac* and different upper-case letters for *M*×*G*. Significant differences in DMC are indicated by bold italic letters, lower-case letters for *MSac* and upper-case letters for *M*×*G*. Level of significance was $\alpha = 0.05$. Error bars represent standard deviation for DMY and DMC.

Table 5 Type 3 test for the significance of main effects and their interactions (genotype, harvest date, genotype*harvest date) on emergence in two genotypes (*MSac* and *MxG*) ($\alpha = 0.05$)

Effect	*F*-value	Pr>*F*
Genotype	5.63	0.0291
Harvest Date	2.22	0.1169
Genotype × Harvest date	0.85	0.4860

Table 6 Emergence rate [%] of collars in the two genotypes (*MSac* and *MxG*) when collars were harvested at four different harvest dates

	Emergence rate (%)	
	Msac	*M*×*G*
08/11/14	66.91 ± 9.171^{ns}	70.26 ± 8.859^{ns}
10/12/14	83.38 ± 6.970^{ns}	90.04 ± 5.491^{ns}
12/01/15	73.39 ± 8.488^{ns}	93.37 ± 4.502^{ns}
12/02/15	76.76 ± 8.057^{ns}	90.07 ± 5.480^{ns}

ns, not significant ($\alpha = 0.05$).

propagation, (ii) the performance of the novel collar propagation method compared to conventional miscanthus propagation methods and (iii) the further development of the collar propagation method for practice.

Suitability of the three lower stem parts for propagation

The results showed that all three lower stem parts, that is collar, rhizome and the combination of collar and rhizome, are suitable for miscanthus propagation.

Where pregrown plantlets were transplanted, no significant differences in establishment and overwintering success were found between the fragments. In contrast, where the fragments were planted directly into the field, the establishment success was significantly higher (depending on genotype) for R+C than for C and R alone (Figs 3 and 4). The R+C fragment is larger and also significantly heavier (Table 1) than the single fragments C and R. It can be assumed that field survival was positively influenced by the weight of the planting material. Previous investigations have observed that shoot emergence rate increases with rhizome weight and size (Christian *et al.*, 2009; Xue *et al.*, 2015). More reserve

substances can be stored in a larger fragment, probably leading to a better overwinter survival of R+C than for single R or C fragments. Therefore, when planting directly into the field, the use of R+C fragments is recommended in order to ensure higher establishment success.

The establishment success of collars was either better than or not significantly different from that of rhizomes when planted directly. Both these fragment types are parts of the stem and had a similar size and weight, except $M\times G$, where fragment C was significantly heavier than fragment R (Table 1). The rhizomes used were only 1 year old and so can be expected to have a better establishment success than older rhizomes. The age of collars is more homogeneous than that of rhizomes, because collars grow annually together with the stems. By contrast, the age of harvested rhizome parts is heterogeneous and depends strongly on the plantation age of the mother field. The vitality and capacity to regenerate a full plant decreases with age of the mother field and thus rhizome age (Christian *et al*., 2009). Therefore, collars are more advantageous, as they are more homogenous in age.

Comparison with other propagation methods

The optimal miscanthus propagation method should be characterized by simplicity, low costs, high reproduction rate of the mother material, low labour and energy inputs, and should ideally be nondestructive for the mother field (Xue *et al*., 2015). Applying these criteria to the results of the four trials of this study, it can be seen that miscanthus propagation via collar fragments is feasible and preferable to conventional rhizome propagation. Harvesting of rhizomes is very labour-intensive, requires heavy machinery and causes soil disturbance, which can lead to soil carbon losses (Boersma & Heaton, 2012) and soil compaction. After harvesting, the rhizomes have to be separated from the roots, cleaned of soil (Xue *et al*., 2015), graded by size and dried out fragments discarded. For this propagation method, viability of the rhizomes is the most important criterion for success and therefore the most important quality criterion for customers purchasing the rhizomes. In addition, it can take up to a maximum of 5 years before sufficient planting material is available in the mother field to be harvested (Christian *et al*., 2005). This means that the harvested rhizomes vary in age, which can influence field emergence. Christian *et al*. (2009) showed that rhizomes from a 9-year-old plantation had a lower viability than rhizomes from a 5-year-old plantation. Therefore, quality screening of the harvested rhizomes is often performed manually to guarantee high-quality rhizomes. This, however, increases the labour intensity of rhizome harvesting.

By comparison, the harvesting of collars is relatively simple. Collars are harvested in a nondestructive way from the mother field by pulling out the stems. As they can be harvested annually, the propagation material is more homogenous than for rhizomes, and the collar fragments can be easily cut to the required size. In addition, harvesting of collars does not disturb the soil, and the remaining mulch layer avoids potential erosion problems. Moreover, collar harvesting delivers fragments of the same age with similar emergence rates, rendering quality screening of viable collars easier than for rhizomes. Future research is required to determine whether, or to what extent, the mother plants are impacted by the harvesting of its collars. If the crop is impacted, a 2-year cycle of collar harvesting may solve the problem. Further research should also clarify whether the age and planting density of the mother field, from which the collars are harvested, influence the viability of collars in different ways.

Establishment success and overwintering losses are also important indicators for the comparison of different propagation methods. In a field trial of Boersma & Heaton (2014a), the establishment loss 2 months after planting reached up to 25% for nodal-stem-propagated plants (transplanted) and up to 34% for (directly planted) rhizome-derived plants. The losses after the first winter were 1–2%. Clifton-Brown *et al*. (2007) also rated establishment survival of micro- and rhizome-propagated $M\times G$ plants in Ireland. At the end of the first growing season, 95% of the plants of both propagation types had survived, whereas after 5 years, 86% of rhizome- and 53% of micro-propagated plants had survived. Although establishment success in our field trial was lower than in those two studies, it was shown that the emergence of collar fragments was as high as for rhizome fragments. As there were virtually no plant losses between the counting at harvest and after winter in Trial 2.2, the overall overwintering survival of the transplanted plants is comparable to the results of Boersma & Heaton (2014a). Apart from *MSac* R+C (70.8%), the plant survival was at a similar level to the results of Clifton-Brown *et al*. (2007), as 87% ($M\times G$ R) to 100% (*MSac* R) of the transplanted plantlets survived until harvest. The higher overwintering losses in Trial 2.3 (15%) were probably caused by the short growing season (June to November) available to the fragments planted directly into the field. This short vegetation period led to lower yields in the directly planted than in the transplanted fragments (Fig. 6). There was obviously not enough time for the plants to establish sufficiently and build up enough reserve substances in the rhizome for regrowth the following year. This can be seen by the plant performance analysed in Trials 2.2 and 2.3. Here, the transplanted plantlets not only had

higher DMY and DMC but, with a few exceptions, also higher stem number and stem diameter (Fig. 5). The transplanted plantlets had more time to develop and, for example produce more stems, and build up enough reserve substances for a regrowth.

As mentioned above, a high multiplication rate is an important factor for an economically successful propagation method. The multiplication rate of collars is lower than that of seed production (>1.500 m^{-2}), and lies more in the region of rhizome propagation (10–50 m^{-2}) (Clifton-Brown *et al.*, 2017). This is due to the fact that every tiller can only deliver one collar. Kalinina *et al.* (2017) found average shoot densities of 29–74 shoots per plant (29 for $M\times G$) with a planting density of 2 plants m^{-2} in various miscanthus genotypes across Europe. Thus, for those genotypes, a harvest of 58–148 collars m^{-2} would be possible if every stem was harvested. However, as this was not analysed in our study, further research is needed to clarify how many stems per plant could be harvested without negatively affecting the mother plant. The multiplication rate of collars is also lower than that of stem cuttings (max. 200 possible; Boersma & Heaton, 2012). However, as described by Xue *et al.* (2015), stem cuttings cannot easily be planted into the field, as they deteriorate rapidly. Therefore, it is better to pregrow them under controlled conditions and then transplant them into the field (Xue *et al.*, 2015). The field experiments described above showed that collars could be planted directly into the field, with some improvements discussed below. When planted directly into the field, collar propagation would be cheaper than stem propagation.

Seeds are easier to store and transport (Clifton-Brown *et al.*, 2017) and need less space than rhizomes and collars. However, propagation via seeds also has some disadvantages compared to collar propagation. First, dedicated seed nurseries need to be established. These are often in warmer regions than the productive miscanthus plantations to allow the seeds to ripen. This means the propagules cannot be produced locally by farmers, leading to further transport costs. In addition, miscanthus seeds are very small, and thus, direct sowing into the fields is not yet a reliable option. Plug plants are currently used instead of sowing seeds directly, but this involves many additional logistical steps, again increasing the costs. Methods for coating seeds to allow direct sowing are still at the experimental stage. The genetic variability of seeds is another problem yet to be solved. Crossing out can lead to genetically inhomogeneous seeds and consequently an inhomogeneous miscanthus stock (Lewandowski *et al.*, 2016). This complicates field management and can result in additional work for farmers as well as lower yields and inhomogeneity of biomass quality. As described above, invasiveness of fertile miscanthus genotypes is also a huge problem. It can result in additional environmental costs, as the native habitat is changed by the invasive grass, and economic costs to curb the invasiveness of the crop (Raghu *et al.*, 2006). Jorgensen (2011) pointed out that sterility is an important goal in future miscanthus breeding to rule out invasiveness before planting. Quinn *et al.* (2010) refer to regulatory restrictions in the United States for certain miscanthus genotypes and therefore recommend sterile or at least functionally sterile genotypes. There are also some other disadvantages of miscanthus propagation via seeds. For example, in areas with low spring temperatures, the earliest possible sowing date may be too late for the crop to develop sufficient rhizome biomass to survive the first winter. Secondly, the risk of overwinter losses increases in plants without fully developed rhizomes (Clifton-Brown *et al.*, 2017). Finally, seed propagation is only feasible for fertile genotypes, mostly *MSin*, and for new hybrids yet to be developed, but a directly sowing is not yet reliable.

To summarize, collar harvest is nondestructive for the mother field, less labour-intensive and therefore cheaper than rhizome propagation. It could become the best propagation method for those miscanthus genotypes that either cannot reproduce via seeds or where genetically homogenous plantations are to be established in temperate regions, using the propagation material from nearby fields.

Development of the collar propagation method in practice

Presently, harvesting of collars has to be performed manually, as no specific machinery is available. Suitable collar harvest machinery needs to be developed to upscale this propagation method. The machine should remove stems and collars from the ground, for example using rubber rollers or a robot arm and, ideally, separate them at the same time.

The harvest trial (Trial 2.4) showed that it is in principle possible to harvest collars throughout the whole winter, as no significant differences were found between the four harvest dates. However, the very early harvest in November had the lowest emergence rate for both genotypes and therefore may be less suitable for commercial application. According to Atkinson (2009), rhizomes are measurably affected by their harvest date, as contents of protein, nitrogen and soluble carbohydrates decrease and contents of lipids increase with a later harvest date. Lipids in particular provide an important energy store for overwintering and regrowth in spring (Atkinson, 2009). Future research therefore needs to determine whether this is also valid for collars, in order to identify the most suitable harvest time.

There were no significant differences in emergence rate of collar fragments from *MSac* and *M*×*G* between the harvest dates December to February. Thus, February harvest is recommended because it reduces storage costs and the drying out of collar fragments, which can reduce establishment success.

Both *MSac* and *M*×*G* showed good establishment success and plant performance when propagated via collar fragments. However, this method does not seem suitable for the genotype of the species *MSin* tested, which can be attributed to the rhizome and collar morphology of *MSin*. This genotype has short, thin rhizomes, whereas *MSac*, and *M*×*G* have thick rhizomes (Lee *et al*., 2012; Xue *et al*., 2015). As described above, thicker rhizomes lead to better establishment of *MSac* and *M*×*G*, as they are able to store more reserve substances. Thus, for these two species tested, propagation via collar fragments is recommended. By contrast, for the tested genotype of the *MSin* species with its thinner rhizomes and fertile seed production, seed propagation is recommended.

The genotypes *MSac* and *M*×*G* showed similar emergence and survival rates for C and R under controlled and field conditions. Therefore, both options, planting collars directly and transplanting pregrown plantlets into the field, may represent opportunities for commercial application of collar propagation. Direct planting of the collar fragments would be preferable, because no additional propagation in the greenhouse is required, saving costs, energy and labour. However, direct planting would necessitate a late collar harvest, for example in April, so that the collars can be planted into the field directly after harvest without any storage. This, however, has not yet been tested and requires further research. To increase establishment success of collars directly planted into the field, one option could be to cover them with a plastic mulch film, which increases soil temperature. O'Loughlin *et al*. (2017) showed that miscanthus rhizomes had a better establishment success, higher stem numbers and higher biomass yields, when they were covered with a plastic mulch film. Another option to improve establishment success and storage suitability of collars could be to encapsulate the collars in a beneficial coating, possibly also including nutrients and growth-promoting substances, to improve emergence rate, as it has been shown for seeds and other propagules (NEF, 2015; Greenfield Mantelsaat®, 2017). Collars could also be coated using such technologies to improve their establishment success when directly planted into the field. The coating would reduce the drying out of the collar fragments, which is one of the main reasons for low emergence rates. In addition, by covering the collars, it may be possible to standardize their size, enabling mechanical planting and thus further reducing establishment costs. Lower establishment costs would also facilitate miscanthus cultivation on marginal land, where establishment is the most challenging phase in the lifetime of a miscanthus crop. Adapting the coating materials to marginal conditions could further enhance establishment success and rooting of the crop in such areas, improving the efficiency of crop production.

An alternative to coating is pregrowing the collars in the greenhouse. In our study, the transplanting of pregrown plantlets resulted in a higher establishment success than the direct planting of collar fragments into the field. However, it should be mentioned that only the stronger plants were transplanted into the fields; we cannot say how the weaker plants would have developed. In practice, it could be possible to transplant the plantlets with a conventional planting machine as used in vegetable production. However, transplanting requires additional production steps, which lead to additional costs, energy and labour and also logistic efforts, as green plantlets have to be shipped. For marginal sites or regions with low temperatures in spring, field establishment via plantlets may be advantageous and result in a higher establishment success.

In conclusion, miscanthus propagation via collars was shown to be viable and a promising alternative to rhizome propagation. Collar propagation enables the generation of homogenous planting material and thus a uniform miscanthus stock. As the harvesting of collars is likely to be less labour-intensive and is less destructive for the mother field than rhizome propagation, this method is more favourable than rhizome propagation for both economic and ecological reasons. However, whereas collar propagation is the most suitable method for the two *MSac* and *M*×*G* genotypes tested, this is not true of the *MSin* genotype. These can already best be propagated by seeds. If collars are directly planted into the field, the fragment R+C should be used. Separated C and R fragments could be used for coated propagation material, which can be easily stored and transported and used for the establishment of homogenous miscanthus plantations, possibly also under marginal site conditions.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Mean shoot emergence over 18 days in the chamber study.

Table S1: Mean shoot emergence (n=6) [in %] over 18 days in the chamber study (Trial 2.1) with the three genotypes (*MSac*, *MxG* and *MSin*) and the three fragment types (collar = C, rhizome and collar = R+C, rhizome = R) with standard deviation

	Shoot emergence [%]								
	MSac C	*MSac* R+C	*MSac* R	*MxG* C	*MxG* R+C	*MxG* R	*MSin* C	*MSin* R+C	*MSin* R
D 2	0±0.00	1±40.76	2±127.74	0±0.00	0±0.00	0±0.00	0±0.00	1±31.42	0±0.00
D 3	5±3.07	17±5.95	28±7.29	4±3.06	14±11.45	0±0.00	5±4.12	8±5.32	0±0.00
D 4	20±6.89	23±7.31	28±7.82	10±5.01	14±9.95	2±2.33	7±5.31	24±10.22	0±0.00
D 5	38±8.50	47±8.74	44±8.70	24±7.89	30±14.31	11±5.26	29±9.77	26±9.32	0±0.00
D 6	42±8.14	53±8.24	44±8.21	26±7.93	53±15.82	14±5.72	39±10.17	30±9.29	3±3.43
D 7	44±8.45	56±8.45	47±8.52	39±9.32	58±15.79	14±5.84	40±10.40	51±10.34	3±3.50
D 8	56±9.21	61±9.02	49±9.32	39±10.19	62±16.72	22±7.72	42±11.43	57±11.05	3±3.79
D 9	58±8.89	64±8.65	55±8.99	44±10.15	79±11.88	25±7.78	48±11.14	54±10.86	6±4.39
D 10	64±9.27	73±8.59	56±9.66	56±10.96	92±6.86	27±8.59	47±12.02	53±11.79	5±4.43
D 11	70±8.66	76±8.05	55±9.48	59±10.64	89±8.77	36±9.14	54±11.89	60±11.43	6±4.84
D 12	70±7.86	76±7.32	55±8.61	65±9.29	93±5.92	36±8.29	53±10.87	59±10.48	6±4.31
D 13	73±7.31	79±6.71	58±8.21	65±8.97	100±0.00	39±8.09	54±10.52	59±10.12	6±4.19
D 14	73±7.30	81±6.32	58±8.20	68±8.74	100±0.00	39±8.07	53±10.59	62±10.02	6±4.07
D 15	73±7.46	84±6.11	61±8.24	72±8.47	100±0.00	39±8.22	54±10.8	59±10.39	8±4.92
D 16	75±7.06	84±6.00	61±8.07	73±8.23	100±0.00	39±8.05	55±10.57	58±10.28	8±4.64
D 17	75±7.06	84±6.00	61±8.079	73±8.23	100±0.00	39±8.05	55±10.57	58±10.28	8±4.64
D 18	75±6.76	84±5.76	62±7.71	79±7.13	100±0.00	39±7.69	54±10.24	57±9.94	8±4.37

3 Reintegration of miscanthus fields into a crop rotation

This chapter deals with the question, how former miscanthus fields can be reintegrated into a crop rotation. For this, a field trial was conducted, in which four different spring crops were grown after a miscanthus stock was removed. This study aimed to answer, which crop could follow miscanthus. Additionally, soil nitrogen analyses were conducted to clarify how the removal effects soil nitrogen content.

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How can miscanthus fields be reintegrated into a crop rotation?

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Abstract

The bioeconomy, with its aim of replacing fossil by biobased resources, is increasingly focusing on biomass production from perennial crops, such as miscanthus. To date, research on miscanthus has explored a number of cultivation aspects; however, one major issue has not yet been addressed: How can former miscanthus fields be reintegrated into a crop rotation? This encompasses the questions of which following crop most efficiently suppresses resprouting miscanthus and what happens to the soil nitrogen content after a miscanthus removal. This study aimed to answer both questions. For this purpose, four spring crops (ryegrass, rapeseed, barley, maize) and fallow as control were cultivated after a *Miscanthus sinensis* removal. To test the effect of the removal on soil nitrogen content, each spring crop (excluding fallow) was divided into fertilized and unfertilized plots. After the spring crop harvest, winter wheat was cultivated to clarify which spring crop had most efficiently suppressed the resprouting miscanthus. The results indicate that fertilized crops had 35% less miscanthus biomass per hectare than unfertilized crops, probably due to the higher plant density and/or better development of the fertilized crops during the growing season. The soil mineral nitrogen (N_{min}) content was found to increase during the vegetation period following the miscanthus removal (average +14.85 kg/ha), but was generally on a low level. We conclude that nitrogen from miscanthus residues is partly fixed in organic matter and is thus not plant-available in the first cropping season. As some nitrogen is supplied by the decomposition of miscanthus residues, our results suggest that the crop cultivated after a miscanthus removal requires less fertilization. Of all the follow-on spring crops tested, maize coped with the prevailing soil conditions and resprouting miscanthus most efficiently, resulting in satisfactory yields, and thus seems to be a suitable crop for cultivation after miscanthus.

KEYWORDS

follow-on crop, maize, miscanthus clearance, nitrogen content, resprouting, ryegrass, spring barley, winter wheat

1 | INTRODUCTION

In the bioeconomy, which aims to replace fossil by biobased resources, there is increasing focus on biomass production by perennial crops. Miscanthus, for example, fulfils several ecological functions, such as soil carbon sequestration and erosion control through its year-round soil coverage and cultivation period of up to 20 years (Lewandowski, Kicherer, & Vonier,

1995). These result in positive effects including improved soil structure and reduced nutrient run-off (McCalmont et al., 2017). Various studies have reported a carbon sequestration under miscanthus in the range of 0.5–2.2 t C ha^{-1} a^{-1} (Blanco-Canqui, 2010; McCalmont et al., 2017). Additionally, miscanthus is high yielding. Schmidt, Lemaigre, Ruf, Delfosse, and Emmerling (2018) have shown that, in temperate climates, yields of between 22 t ha^{-1} a^{-1} (brown harvest after winter) and 27 t ha^{-1} a^{-1} (green harvest in autumn) can be achieved.

It is these high yields together with environmentally benign characteristics that render miscanthus a promising bioeconomy crop with a number of utilization options. Currently, its most common utilization pathway is combustion (Iqbal & Lewandowski, 2016). However, other potential pathways include ethanol production (van der Weijde et al., 2017), anaerobic digestion (Mangold et al., 2018; Mayer et al., 2014) and building materials.

Most recent studies on miscanthus have focused on its improvement for those utilization pathways or dealt with agronomic aspects such as establishment options (Boersma & Heaton, 2014; Clifton-Brown, Hastings, & Mos, 2017; Xue, Kalinina, & Lewandowski, 2015), general cultivation practices including row distance and fertilization (Larsen, Jørgensen, Kjeldsen, & Lærke, 2014), or examined the performance of various genotypes on different site conditions (Lewandowski et al., 2016).

Although miscanthus has been explored from these different perspectives, one main research question has to date been almost neglected: How can former miscanthus fields be reintegrated into rotations with annual crops?

This question of the reintegration or removal of former miscanthus fields has only been dealt with in a few studies. McCalmont et al. (2018), for example, investigated the nitrous oxide emissions after a miscanthus removal. Dufossé, Drewer, Gabrielle, and Drouet (2014) analysed the effect of a miscanthus removal on soil nutrient stock, greenhouse-gas emissions and the yield of the following crop (wheat). Drewer, Dufossé, Skiba, and Gabrielle (2016) examined the effect of a removal on the isotopic signature of soil carbon.

In the three studies mentioned above, miscanthus was removed by the application of glyphosate. However, glyphosate application is currently the subject of controversial debate as negative environmental impacts are expected (Myers et al., 2016). Therefore, the question arises as to how miscanthus can be cleared from the field at the end of its lifetime without using glyphosate. This question is crucial for farmers, as the resprouting of miscanthus through its rhizomes may impair follow-on crops. The problem can be seen in the example of *Elymus repens* L., a rhizomatous perennial weed, which can cause high yield losses in crops, but can be successfully removed by glyphosate application (Ringselle, Bergkvist, Aronsson, & Andersson, 2015). Ringselle et al. (2015) showed that soil tillage or covering by crops can be a nonchemical alternative to reduce *E. repens*. Therefore, the question arises whether tillage and the cultivation of follow-on crops can also suppress resprouting miscanthus.

An additional important question is the effect of a previous crop on soil nitrogen (N) content. In Germany, this has become particularly important since the recent release of the amendment to the Fertiliser Application Ordinance (Bundesministerium für Ernährung und Landwirtschaft (BMEL), 2019). Today, there is still little information available on the effect of miscanthus cultivation and its subsequent removal on soil N content. It is well known that the removal of permanent grassland leads to an increase in soil N content (Seidel, Kayser, Müller, & Isselstein, 2009). An increase is to be expected when both living and dead plant material as well as soil organic matter are mineralized (Seidel et al., 2009). This, in turn, can lead to nitrate leaching (Seidel et al., 2009). A removal of permanent crops, such as grassland or miscanthus, may also lead to high nitrous oxide emissions (Dufossé et al., 2014; McCalmont et al., 2018; Pinto et al., 2004; Vellinga, Pol-van, & Kuikman, 2004).

On the other hand, it is known from cereal cultivation that if straw (which has high carbon content) is left on the field and the soil N content is low, the following crops are negatively affected as soil bacteria use up the N for the decomposition of the straw (Reinertsen, Elliott, Cochran, & Campbell, 1984). This may also be the case after a miscanthus removal, as rhizomes and litter remain on the field. If N content is low and the follow-on crop is not adequately fertilized, it may suffer from N shortage.

It is currently unclear which effect is to be expected in the first year after a miscanthus removal: high N availability as observed after a grassland removal or a possible N shortage as in some cases of cereal cultivation.

The aim of this study was to answer both questions mentioned above. (a) Can resprouting miscanthus be suppressed by soil tillage and the cultivation of follow-on crops? (b) What is the soil N availability for a follow-on crop after a miscanthus removal?

2 | MATERIALS AND METHODS

2.1 | Field trial

The field trial was conducted at the University of Hohenheim's research station 'Ihinger Hof' (48.7° latitude, 8.9° longitude, approximately 475 m a.s.l.). The location is characterized by a long-term average annual air temperature of 9.5°C and an annual precipitation of 720 mm. The soil is classified as luvisol (soil type: loam; pH: 6.9) and soil nutrient content of both P and K was classified as 'C' according to VDLUFA (Association of German Agricultural Analytic and Research Institutes) soil classification. The weather conditions during the field trial, shown

on a monthly basis from February 2017 to July 2018, can be found in Table S1.

The miscanthus genotype OPM-111 (polycross of parents selected in Indiana, US from five *M. sinensis* accessions collected in central Japan) was planted in May 2013 and, after 4 years of cultivation, removed by ploughing. As the miscanthus stock was inhomogeneous, the density was determined by counting plants/m^2 in each plot on 15 December 2016 to test whether there were significant differences between the plots before the removal was carried out.

The miscanthus field was harvested on 15 February 2017 using a field chopper. Two weeks later, the field was ploughed to a depth of 20 cm and harrowed with a rotary power harrow. Afterwards, a split-plot design was used to allocate spring crops and fertilization treatments to the field. All variants of the follow-on spring crops (annual ryegrass, spring barley, summer rapeseed, maize; each both fertilized and unfertilized) were then sown in strip plots in a randomized complete block design with four replicates (Figure 1). The strip plots of the spring crops (main factor) were randomly divided into unfertilized and commonly fertilized subplots. The plot size

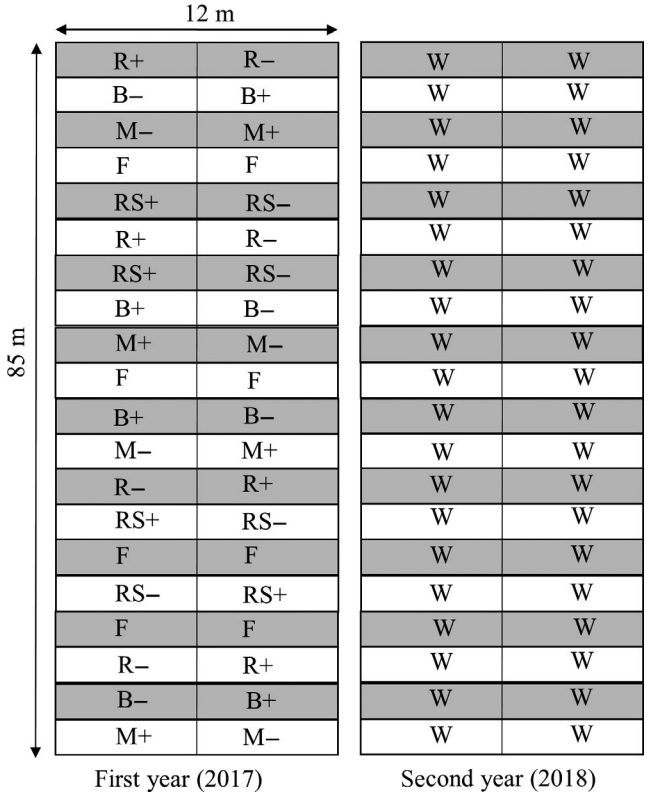

FIGURE 1 Field trial design: After miscanthus removal in February 2017, four spring crops (R: ryegrass; B: barley; M: maize; RS: rapeseed) were cultivated and managed by 'good agricultural practice' with (+) or without fertilization (−). Additionally, as control, some plots stayed fallow (F). Following harvest of the spring crops in 2017, winter wheat (W) was sown on all plots in autumn 2017 as second vegetation after miscanthus and managed by good agricultural practice throughout the trial period

was 18 m^2 (6 m × 3 m) for ryegrass (*Lolium perenne* L.), barley (*Hordeum vulgare* L.), rapeseed (*Brassica napus* L.) (fertilized/unfertilized), and the control fallow (unfertilized) and 27 m^2 (6 m × 4.5 m) for maize (*Zea mays* L.) (fertilized/unfertilized), where it was adopted to the working width of the sowing machine. Details of the spring crop cultivation are given in Table 1.

The rapeseed stock was inhomogeneous and weeds dominated each plot, probably due to the low temperatures in April (down to −5°C on 20 April 2017). For this reason, we decided to omit rapeseed from the N analysis.

For the harvest of all spring crops and fallow, 2 m^2 was cut from the middle of each plot and the harvested biomass was then divided into the fractions given in Table 1. The entire biomass in the 2 m^2 was harvested with an electric cutter at a cutting height of 5 cm. The biomass was then transported to the laboratory for separation. It was weighed, chopped and dried at 60°C to constant weight to estimate the dry mater content (DMC). From the DMC, the dry matter yield (DMY) was calculated. The barley was dried as whole crop and then threshed to determine grain and straw yield.

After the harvest of the 2 m^2, the rest of the biomass from each plot was cut on the same day and discarded.

In autumn 2017, all plots were ploughed and, 3 days later, sown with winter wheat. Details of winter wheat cultivation are given in Table 2.

At the winter wheat harvest, the same 2 m^2 as in the previously grown spring crops were cut, supported by GPS tracking and separated into wheat and miscanthus. The biomass fractions were weighed and dried at 60°C to constant weight for DMC estimation. The wheat was then threshed to determine straw and grain yields. The DMY of grain, straw and, where present, miscanthus was calculated from the DMC.

Figure 1 gives a schematic overview of the field trial for the 2 years 2017 and 2018.

2.2 | Nitrogen content of soil and crop

The soil N_{min} (NO_3-N) content was analysed (following guideline VDLUFA A 6.1.4.1) in each plot twice: after the miscanthus harvest/before sowing of spring crops (20 February 2017) and after the spring crop harvest (16 October 2017). Soil samples were taken at three depths (0–30, 30–60 and 60–90 cm). For N_{min} content determination, a $CaCl_2$ extraction was performed with the fresh soil and the N_{min} measured using a flow injection analyser. Afterwards, N_{min} content per hectare was calculated using a bulk density of 1.4 g/cm^3.

Nitrogen contents of the follow-on spring crops ryegrass (first and second cut), barley (grain and straw) and maize (whole plant) were analysed using a Vario Max CNS (Elementar Analysensysteme GmbH, Langenselbold, Germany), as described in the VDLUFA Method Book III, method 4.1.2 and DIN ISO 5725.

TABLE 1 Overview of spring crop cultivation

Spring crop	Sowing	Fertilization	Crop protection	Harvest
Ryegrass +N	16 March 2017 45 kg/ha variety 'Pollanum' Sown with a seed drill combination Rolled 1 day later using a front roller	24 April 2017 first fertilization 6 July 2017 second fertilization Each time: 50 kg N/ha via calcium ammonium nitrate (27% N)	None	4 July 2017 first harvest[a] 20 September second harvest[a] Each time biomass divided into fractions 'ryegrass' and 'miscanthus' Weeds assigned to ryegrass, as only present in small amounts
Ryegrass −N	23 May 2017 cut to stimulate growth (grass was removed)	None		
Barley +N	16 March 2017 168 kg/ha variety 'RGT Planet' (malting barley) Sown with a seed drill combination Rolled 1 day later using a front roller	24 April 2017 70 kg N/ha via calcium ammonium nitrate (27% N)	4 May 2017 herbicides 'Axial' 50 (0.9 L/ha; Syngenta; components: pinoxaden, cloquintocet) 'Alliance Suprim' (0.1 kg/ha; Nufarm; components: diflufenican, metsulfuron) 8 June 2017 Fungicides 'Aviator Xpro' (0.6 L/ha; Bayer; components: bixafen, prothioconazole) 'Fandango' (0.6 L/ha; Bayer; components: prothioconazole, fluoxastrobin) Insecticide 'Lambda WG' (150 g/ha; Syngenta; component: lambda-cyhalothrin)	24 July 2017 Biomass divided into fractions 'barley', 'miscanthus' and 'weeds'
Barley −N		None		
Rapeseed +N	27 March 2017 4.78 kg/ha variety 'Makro' Sown with a seed drill combination Rolled 1 day later using a front roller	24 April first fertilization (70 kg N/ha) 14 June 2017 second fertilization (50 kg N/ha) Each time via calcium ammonium nitrate (27% N)	4 May 2017 herbicide 'Butisan'(1.5 L/ha; BASF; component: metazachlor) 14 June 2017 insecticide 'Biscaya' (0.3 L/ha; Bayer; component: thiacloprid)	20 September 2017 Biomass divided into fractions 'miscanthus' and 'remaining biomass', which included rapeseed (whole crop due to inhomogeneous rapeseed stocks) and weeds
Rapeseed −N		None		
Maize +N	17 May 2017 Plots harrowed with rotary power harrow 18 May 2017 93,200 grains/ha variety 'Ridley' Sown with a pneumatic single grain seeder	22 May 2017 first fertilization (120 kg N/ha) 14 June 2017 second fertilization (120 kg N/ha) Each time via calcium ammonium nitrate (27% N)	13 June 2017 herbicides MaisTer Power (1.4 L/ha; Bayer; components: foramsulfuron, thiencarbazone, iodosulfuron, cyprosulfamide) Bromotril 225 EC (0.3 L/ha; Adama; component: bromoxynil)	20 September 2017 Biomass divided into fractions 'miscanthus' and 'maize' Weeds were neglected due to their small amount
Maize −N		None		
Fallow	None	None	None	20 September 2017 Biomass divided into the fractions 'miscanthus' and 'remaining biomass'

[a]The same 2 m^2—tracked by GPS—were harvested on both harvest dates.

TABLE 2 Overview of winter wheat cultivation

Sowing	16 October 2017 157 kg/ha variety 'Rebell' Sown with a seed d'ryegrass;rill combination
Fertilization	3 April 2018 first fertilization 150 kg/ha urea (69 kg N/ha) 3 May 2018 second fertilization (70 kg N/ha) 24 May 2018 third fertilization (40 kg N/ha) Second and third fertilization via calcium ammonium nitrate (27% N)
Crop protection	24 April 2018 herbicides 'Atlantis' (330 g/ha; Bayer; components: mesosulfuron, odosulfuron) +formulation enhancer (600 ml/ha; Bayer; component: mefenpyr) 'Alliance' (90 g/ha; Nufarm; components: diflufenican, metsulfuron) 'Tomigan' (0.5 L/ha; ADAMA; components: fluroxypyr, florasulam) Growth regulator 'Cycocel' (0.6 L/ha; BASF; component: chlormequat chloride) 29 May 2018 Insecticide 'Lambda WG' (150 g/ha; Syngenta; component: lambda-cyhalothrin) Fungicides 'Credo' (1.25 L/ha; DuPont; components: picoxystrobin; chlorothalonil) 'Input Classic' (0.75 L/ha; Bayer; components: prothioconazole, spiroxamine)
Harvest	24 July 2018 Biomass divided into fractions 'miscanthus' and 'wheat' (Weeds were neglected due to their small amount)

2.3 | Statistical analysis

Data analysis was performed using the PROC MIXED procedure of Statistical Analysis Software SAS, version 9.4 (SAS Institute Inc.). Normal distribution and homogeneous variance of residuals were checked graphically.

For the statistical analysis of miscanthus regrowth, DMY of spring crops and winter wheat, the model given in Equation (1) was used. Crop, fertilization and their interactions were taken as fixed effects. The effect of miscanthus density determined before crop removal was taken as covariable. It was found to be nonsignificant in each test.

$$y_{ihk} = \mu + g_i + d_h + (gd)_{ih} + fx_{ihk} + s_k + (sg)_{ik} + e_{ihk}, \quad (1)$$

where y_{ihk} is the measurement for the i-th crop on the h-th fertilization level in the k-th field replicate. μ is the general effect, g_i is the i-th crop effect (ryegrass; barley, maize, fallow land; rapeseed), d_h is the main effect of the h-th fertilization level (fertilized; unfertilized), $(gd)_{ih}$ is the interaction effect of the i-th crop with the h-th fertilization level, f is the slope of miscanthus density x_{ihk} before removal and s_k is the random effect of the k-th replicate in the field. e_{ihk} and $(sg)_{ik}$ are the residual and main plot error terms corresponding to y_{ihk} and the ik_{th} main plot.

For the N content, the model is slightly more complicated, as (a) ryegrass was cut twice and each cut analysed separately; and (b) barley was subdivided into grain and straw and each part analysed separately. Therefore, g_i is split into g_i and $(gm)_{il}$ or g_i and $(gn)_{io}$ with m_l being the effect of the l-th harvest date and n_o being the effect of the o-th effect of plant part.

$$y_{ihlok} = \mu + g_i + d_h + (gd)_{ih} + fx_{ihk} + (gm)_{il} + (gdm)_{ihl} + (gn)_{io} + (gdn)_{iho} + s_k + (sg)_{ik} + e_{ihlok}. \quad (2)$$

For the soil N_{min} content, soil sampling date (p) was added to Equation (1) to give Equation (3).

$$y_{ihqk} = \mu + g_i + d_h + p_q + (gd)_{ih} + (gp)_{iq} + (dp)_{hq} + (gdp)_{ihq} + fx_{ihk} + s_k + (sg)_{ik} + e_{ihqk}, \quad (3)$$

where p_q is the q-th soil sampling date (23 February 2017 or 16 October 2017).

Effects of field replicates were assumed to be random. Multiple t tests with a significance level of $\alpha = .05$ were conducted only where differences were found via a global F test. In the figures, a letter display was used where identical letters indicate that means are not significantly different from each other.

3 | RESULTS

3.1 | Resprouting miscanthus in follow-on spring crops

The average miscanthus density before clearance was 0.8 plants/m^2 (Figure 2a). The highest density was found before ryegrass +N (0.96 plants/m^2) and the lowest before ryegrass −N (0.66 plants/m^2). As mentioned above, miscanthus density was taken as a covariable in each statistical analysis and, in each analysis, was found to have no significant effect (see Table 3).

Figure 2b shows the average number of miscanthus stems, counted in July 2017, in the follow-on spring crops. As shown in Table 3, crop (0.0032) and fertilization (0.0005) had a significant effect on resprouting, but their interaction did not. The lowest number of miscanthus stems was found in barley +N (0.61 stems/m^2), although the former miscanthus plant density was quite high (0.85 plants/m^2). The highest number of miscanthus stems was found in rapeseed −N (12.24 stems/m^2) (Figure 2b), where the former miscanthus plant density

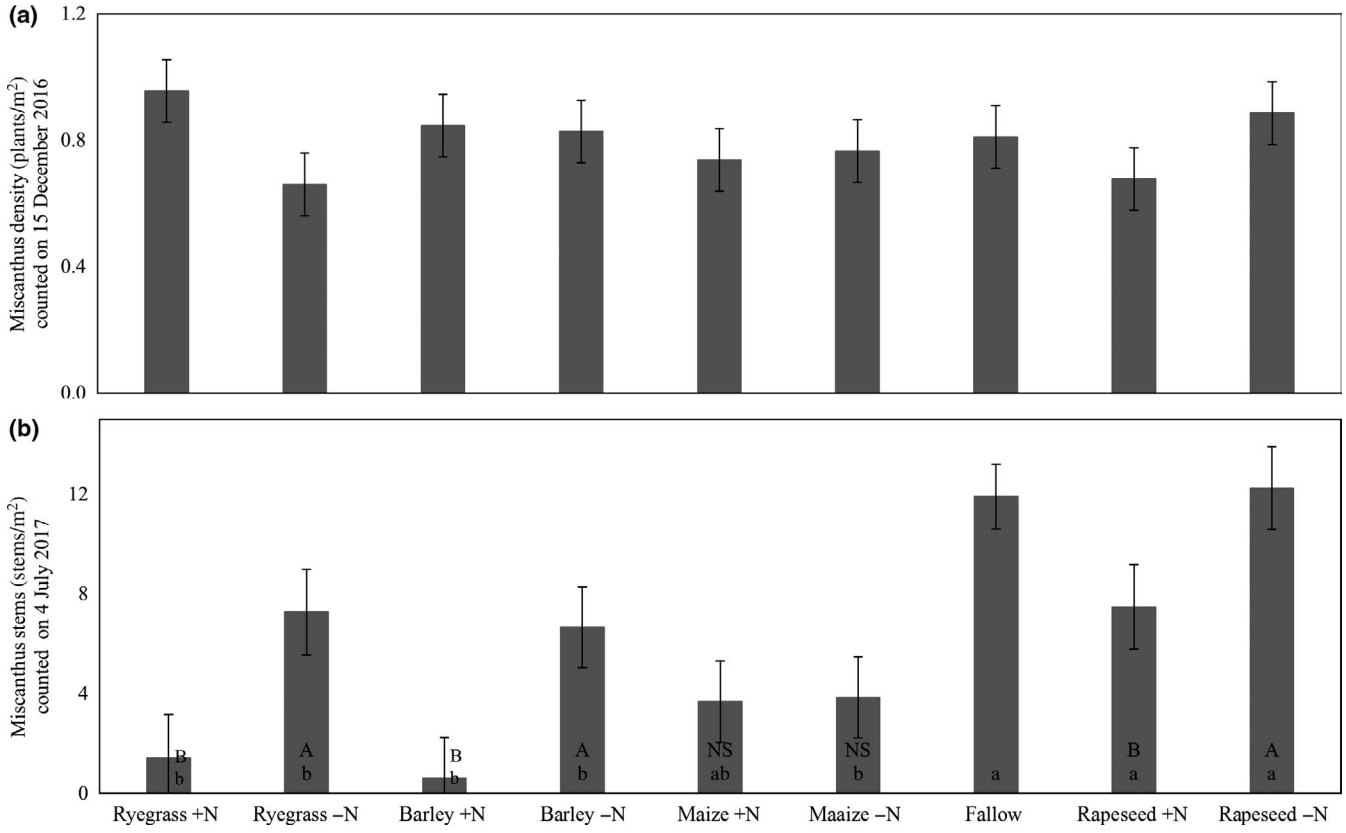

FIGURE 2 Overview of miscanthus resprouting in follow-on spring crops: (a) average density of miscanthus plants (plants/m^2) for each plot, counted on 15 December 2016 before miscanthus removal. (b) Number of resprouted miscanthus stems (average values) in the spring crops, counted in July 2017. Different lower-case letters indicate significant differences in resprouting miscanthus between spring crops (fertilized crops are compared with each other; unfertilized crops including fallow are compared with each other). Different upper-case letters represent significant differences between the fertilized and unfertilized variant of the same spring crop (NS = not significant). Means with the same letter were not significantly different from each other. Error bars show standard error, level of significance was $\alpha = .05$

TABLE 3 *p* Values for *F* tests of fixed effects ($\alpha = .05$) for resprouted miscanthus and dry matter yields (DMY) of spring crops and winter wheat

	Crop	Fertilization	Crop × Fertilization	Miscanthus density
No. of miscanthus stems in July 2017	0.0032	0.0005	0.1516	0.4859
DMY of spring crops 2017	<0.0001	<0.0001	<0.0001	0.8760
Miscanthus in spring crops 2017	0.0002	0.0809	0.5944	0.1538
DMY of wheat grain 2018	0.1671	0.6652	0.3523	0.9316
Miscanthus in wheat 2018	0.0072	0.4937	0.8956	0.6567
Nitrogen content of spring crop biomass 2017	0.0015	0.7838	0.0007	0.4419

(0.89 stems/m^2) was similar to that of barley +N. Comparing fertilized with unfertilized crops, (significantly) less stems were found in fertilized than in unfertilized plots for each crop except for maize (see upper-case letters in Figure 2b). The miscanthus resprouting in the follow-on crops can be seen in Figure 3.

FIGURE 3 Resprouting miscanthus in follow-on spring crops after miscanthus removal in annual ryegrass (a), spring barley (b), summer rapeseed (c), maize (d) and fallow (e) in 2017. (f) shows the resprouting miscanthus in winter wheat, sown in autumn 2017 after harvest of follow-on spring crops (a–e)

TABLE 4 Dry matter yield (DMY) and biomass amount (dry matter = DM) of spring crops, miscanthus and winter wheat. Yield/biomass amount is shown in kg/ha for miscanthus and in t/ha for all other crops. Different upper-case letters indicate significant differences between fertilized and unfertilized variant of same (previous) spring crop (NS = not significantly different, $\alpha = .05$). Different lower-case letters indicate significant differences between spring crops (fertilized crops are compared with each other; unfertilized crops including fallow are compared with each other)

	DMY (t/ha)	Biomass amount (DM), kg/ha	DMY, t/ha	Biomass amount (DM), kg/ha
(Previous) spring crop	Spring crop	Miscanthus (in spring crop)	Winter wheat (grain)	Miscanthus (in winter wheat)
Ryegrass +N	$3.95 \pm 1.20^{NS,*}$	$130 \pm 320^{b,*}$	5.00 ± 0.48	4.92 ± 4.09^{ns}
Ryegrass −N	$2.47 \pm 1.20^{NS,*}$	$870 \pm 320^{bc,*}$	5.68 ± 0.48	4.20 ± 4.79^{b}
Barley +N	2.54 ± 1.17^{A} (grain)	70 ± 310^{b}	5.43 ± 0.45	0.00 ± 3.90^{ns}
Barley −N	0.69 ± 1.16^{B} (grain)	380 ± 310^{c}	5.63 ± 0.45	0.78 ± 3.90^{b}
Maize +N	26.92 ± 1.17^{A} (whole crop)	360 ± 310^{b}	6.57 ± 0.45	2.10 ± 3.91^{ns}
Maize −N	17.54 ± 1.17^{B} (whole crop)	390 ± 310^{c}	5.72 ± 0.45	7.60 ± 3.89^{ab}
Fallow	1.31 ± 1.10 (total biomass)	$1{,}480 \pm 250^{ab}$	5.22 ± 0.31	17.34 ± 2.75^{a}
Rapeseed +N	2.50 ± 1.19^{NS} (whole crop ± weeds)	$1{,}760 \pm 320^{a}$	5.41 ± 0.46	1.34 ± 4.00^{ns}
Rapeseed −N	0.97 ± 1.18^{NS} (whole crop ± weeds)	$1{,}930 \pm 310^{a}$	4.89 ± 0.46	3.42 ± 3.95^{b}

*Average of first and second cut; the same 2 m^2—tracked by GPS—were harvested on both harvest dates.

3.2 | Yield of spring crops and winter wheat, including miscanthus biomass

At the harvest of each follow-on spring crop, the biomass amounts of both the crop and miscanthus were determined. As the rapeseed developed poorly, all biomass growing on the plot was harvested with the rapeseed being harvested as whole crop (not, as usual, only the seeds).

Table 4 gives an overview of the DMY of each follow-on spring crop. Fertilization and the interactions of Fertilization × Crop had a significant effect on the DMY (see Table 3) and resulted in higher DMY for fertilized than unfertilized spring crops. For maize and barley (grain), this effect resulted in a significantly higher DMY for the fertilized than the unfertilized variant (see upper-case letters Table 4).

The amount of miscanthus in the follow-on spring crops was significantly affected by crop (Table 3). With an average of 1,840 kg DM/ha, rapeseed had the significantly highest amount of miscanthus in all fertilized and unfertilized crops (see lower-case letters in Table 4). The lowest amount of miscanthus biomass was found in barley +N (70 kg/ha) (see Table 4). Fertilization resulted in lower amounts of miscanthus in all crops, but this effect was not significant. The average amount of miscanthus was 580 kg/ha in fertilized crops and 890 kg/ha in unfertilized crops (excluding fallow).

The DMY of the winter wheat (grain) cultivated after the spring crops was not significantly affected by the (previous) crop, fertilization or the interaction of both (see Table 3). The DMY of wheat (grain) for each previous crop is shown in Table 4.

In general, the amount of resprouting miscanthus in winter wheat was low (average: 4.63 kg/ha). It was significantly affected by the previous crop (Table 3). The highest amount of miscanthus biomass was found on previously fallow land (17.34 kg DM/ha, Table 4). The amount of miscanthus biomass was lower in all previously fertilized plots (2.09 kg/ha) than unfertilized plots (4.0 kg/ha, excluding fallow), except for ryegrass. The previous barley +N plots were entirely free of miscanthus biomass (Table 4).

3.3 | Soil N_{min} content and N content of spring crops

Table 5 gives the *p*-values for *F* tests of fixed effects for each soil depth and also for the total (0–90 cm). Table 6 shows the N_{min} contents (NO_3-N) of the three depths (0–30, 30–60, 60–90 cm) and the total N_{min} (0–90 cm) determined after the miscanthus harvest (d1; 23 February 2017) and after the spring crop harvest (d2; 16 October 2017). The average N_{min} contents measured on both d1 (2.4 kg/ha) and d2 (17.27 kg/ha) were generally low. N_{min} content increased significantly from d1 to d2 in all unfertilized plots at each soil depth (see Table 5). This is particularly visible in fallow land, which was not fertilized: the N_{min} amount increased from 2.0 kg N_{min}/ha in the first assessment to 38.71 kg N_{min}/ha in the second (Table 6). This was the highest N_{min} amount found in all plots, both fertilized and unfertilized. The higher N_{min} contents of unfertilized than fertilized crops at d2 (for example in ryegrass, barley, rapeseed 0–30 cm) were not significant and probably reflect a natural fluctuation.

In addition, Table 5 shows that fertilization and miscanthus density before removal did not significantly affect the soil N_{min} content. In Table 6, there is no letter display, as significant differences were only found for soil sampling date and between different (fertilized) crops (which was expected and is thus not shown by letters) but not for fertilization itself (Table 5), which was the focus of our study. (Differences between unfertilized crops only occurred for fallow, which had a significant higher N_{min} content than other crops at 0–30 and 0–90 cm.)

Figure 4 shows the N content of the spring crops ryegrass (first and second cut), barley (grain and straw) and maize (whole crop). As shown in Table 3, crop and the interactions of crop with fertilization had a significant effect on the N content, shown by the letter display in Figure 4. Fertilization itself, however, did not have a significant effect on the N content.

In ryegrass, the N content was higher in unfertilized (1.4% of DM; average of harvest date (HD)1 and HD2) than fertilized (1.2% of DM; average of HD1 and HD2) crops, but this was only significant at HD2. A comparison of the two

TABLE 5 *p* Values for *F* tests of fixed effects ($\alpha = .05$) for soil N_{min} content of samples taken on 23 February 2017 and 16 October 2017, at three soil depths (0–30; 30–60; 60–90 cm) and in total (0–90 cm)

Depth in cm	Crop	Fertilization	Date	Crop × Fertilization	Crop × Date	Fertilization × Date	Crop × Fertilization × Date	Density
0–30	0.0069	0.3273	<0.0001	0.0091	0.0087	0.0954	0.0040	0.0735
30–60	0.3255	0.7520	0.0004	0.9155	0.1153	0.9656	0.9482	0.8503
60–90	0.1287	0.7861	<0.0001	0.6355	0.0093	0.7277	0.5582	0.9332
0–90	0.0332	0.7455	<0.0001	0.3554	0.0011	0.4341	0.3309	0.6071

TABLE 6 Average N_{min} (NO_3-N) content (kg N_{min}/ha) of soil after miscanthus removal/before sowing of spring crops (d1; 23 February 2017) and after harvest of spring crops (d2; 16 October 2017) at three soil depths (0–30; 30–60; 60–90 cm) and in total (0–90 cm) for fertilized (+N) and unfertilized (−N) plots of spring crops

	kg N_{min}/ha							
	0–30 cm		30–60 cm		60–90 cm		Total 0–90 cm	
	d1	d2	d1	d2	d1	d2	d1	d2
Ryegrass +N	1.01 ± 1.7	4.25 ± 1.7	0.81 ± 3.8	2.72 ± 2.6	0.65 ± 3.6	8.05 ± 2.6	1.91 ± 4.9	15.02 ± 4.9
Ryegrass −N	1.01 ± 1.7	4.78 ± 1.7	0.84 ± 2.7	2.72 ± 2.7	0.81 ± 2.6	2.70 ± 2.6	2.67 ± 4.9	10.26 ± 4.9
Barley +N	0.96 ± 1.7	4.43 ± 1.7	0.50 ± 2.6	3.95 ± 2.6	1.21 ± 2.6	1.87 ± 2.6	2.66 ± 4.9	10.23 ± 4.9
Barley −N	1.23 ± 1.8	6.14 ± 1.8	1.30 ± 2.6	7.11 ± 2.6	1.05 ± 2.6	1.21 ± 2.6	3.56 ± 5.0	18.97 ± 5.0
Maize +N	0.85 ± 1.7	17.55 ± 1.7	0.33 ± 2.6	4.98 ± 2.6	0.71 ± 2.6	2.13 ± 2.6	1.88 ± 4.9	24.66 ± 4.9
Maize −N	1.88 ± 1.7	4.04 ± 1.7	0.86 ± 2.6	3.67 ± 2.6	0.39 ± 2.9	1.96 ± 2.6	3.21 ± 4.9	9.67 ± 4.9
Fallow	1.01 ± 1.3	10.06 ± 1.2	0.57 ± 2.1	12.69 ± 1.9	0.31 ± 2.7	15.97 ± 2.0	2.0 ± 3.7	38.71 ± 3.7
Rapeseed +N	0.64 ± 1.8	3.02 ± 1.8	0.50 ± 2.7	3.57 ± 2.7	0.52 ± 3.8	7.18 ± 2.7	1.40 ± 5.1	13.74 ± 5.1
Rapeseed −N	1.45 ± 1.8	4.87 ± 1.8	0.64 ± 3.8	3.65 ± 2.7	1.14 ± 3.0	5.70 ± 2.7	2.61 ± 5.1	14.25 ± 5.1

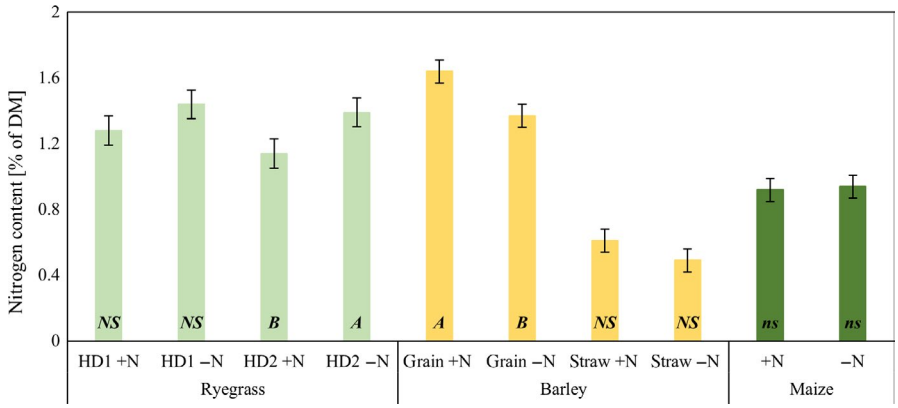

FIGURE 4 Nitrogen content (% of DM) of biomass from the three spring crops ryegrass (first harvest date (HD1) and second HD2 cut), spring barley (grain and straw), and maize (whole crop), for both fertilized (+N) and nonfertilized (−N) variants. Error bars represent standard error. Letter display indicates significant differences between fertilized and unfertilized variant of same previous spring crop for same harvest date or plant part (NS = not significantly different, $\alpha = .05$)

cuts (average of fertilized/unfertilized crops) showed the N content of the first cut (1.36% of DM) to be higher than the second (1.26% of DM). In barley, the N content of fertilized crops (grain: 1.64% of DM; straw: 0.61% of DM) was higher than for unfertilized crops (grain: 1.37% of DM; straw: 0.49% of DM) (see Figure 4). In maize, the N content was similar in unfertilized (0.94% of DM) and fertilized crops (0.91% of DM) (Figure 4).

Using DMY to calculate the N removed by the harvested biomass gives a higher N removal for fertilized than unfertilized plots for ryegrass, barley and maize, due to the overall higher yield. Maize had the highest removal at, on average, 206 kg N/ha. This was followed by ryegrass which removed on average 25 kg N/ha (first cut) and 17 kg N/ha (second cut). Barley had an average N removal of 26 kg/ha (grain) and 5 kg/ha (straw).

4 | DISCUSSION

This study analysed how former miscanthus fields can be reintegrated into annual crop rotations. The aim was to determine whether resprouting miscanthus can be suppressed by soil tillage and the cultivation of follow-on annual crops and what happens to soil N content after miscanthus removal. The results showed that the miscanthus stand was successfully cleared by the follow-on cultivation of ryegrass, barley or maize. In addition, it was shown that, after the miscanthus removal, mineral nitrogen (N_{min}) content increased significantly from the first to the second soil sampling, not only in fertilized but also in unfertilized plots. However, the N_{min} contents were generally on a low level after the miscanthus removal.

The following sections discuss (a) the effect of crop management and crop competition on the suppression of resprouting miscanthus; and (b) N management after a miscanthus removal.

4.1 | Effect of crop management and crop competition on resprouting of miscanthus

The results of this study show that less miscanthus regrew in the fertilized than unfertilized follow-on spring crops, although this effect was not significant. This trend also carried through to the winter wheat cultivated in the next season, except for the previous ryegrass +N plot. This was most likely due to the higher vigour of fertilized than unfertilized crops, resulting in a higher plant density and/or better development of plant stocks over the growing season, as it is well known that a higher vigour of plants leads to more efficient weed suppression (e.g. Bertholdsson, 2005).

Another finding of the study was that barley +N had the lowest miscanthus regrowth on all three assessment dates (during growing season and at harvest of spring crops; at harvest of winter wheat). The highest miscanthus biomass was found in rapeseed (+/− N) and in fallow land. Due to the poor establishment and low crop density of rapeseed, its effect in suppressing resprouting miscanthus was not much different to that of fallow land.

A comparison of the three crops maize, barley and ryegrass reveals how two different 'mechanisms' of miscanthus suppression become relevant: the effect of 'crop management' on the one hand and of 'crop competition' on the other.

In maize, both mechanisms applied. Initially, the mechanism 'crop management' took effect as maize was sown latest of all crops. The miscanthus that had sprouted up until the sowing of maize was removed mechanically by a second harrowing of the field before sowing. Thus, the miscanthus rhizomes were first reduced to smaller pieces, resulting in a lower resprouting rate (Isensee, Ohls, & Quas, 1994). Secondly, the harrowing brought the rhizomes up to the soil surface and the consequent drying out also hindered resprouting (Isensee et al., 1994). However, once the maize was sown, the miscanthus was able to develop until the maize had sprouted and covered the whole soil surface. Later, when the maize canopy had closed, the mechanism of 'crop competition' came into effect: the miscanthus was efficiently suppressed, as the greater plant height of maize enabled it to intercept the photosynthetically active radiation more successfully (Bertholdsson, 2005). This resulted in a comparatively low number of miscanthus stems (counted on 4 July) and ultimately a low proportion of miscanthus in the total biomass at harvest, mainly due to the high biomass yield of maize (up to 26.92 t DM/ha for maize +N).

In ryegrass, the mechanism of 'crop management' contributed to the efficient removal of miscanthus. Its resprouting was impaired by mowing the ryegrass three times: at the growth-stimulating cut in mid-May and the two harvests in July and September. After each cut, the miscanthus started to regrow, finally leading to its exhaustion (Isensee et al., 1994). As shown by Ringselle et al. (2015), *E. repens* can be reduced by 50% through the cultivation of ryegrass if this is cut twice. Our study showed that miscanthus regrowth can also be sufficiently suppressed by mowing. The average proportion of miscanthus in total biomass was 6.8% for ryegrass +N and 23.7% for ryegrass −N. However, the amount of miscanthus biomass increased from the first to the second harvest (+230 kg DM/ha in ryegrass +N; +540 kg DM in ryegrass −N). This is most likely due to the lower ryegrass yield, which decreased from the first to the second harvest in both variants (−0.5 t DM/ha for ryegrass +N; −0.45 t DM/ha for ryegrass −N), leading to a less efficient suppression of miscanthus. As the ryegrass plots were cut three times (growth-stimulating cut, first and second harvest), we would have expected the amount of miscanthus biomass to decrease. Therefore, further research should clarify whether the timing of the mowing is also important and affects miscanthus regrowth, as mentioned by Ringselle et al. (2015) for *E. repens*.

In barley, the mechanism of 'crop competition' was predominantly effective. Barley was able to prevent the resprouting of miscanthus by its stronger competition for light, water and nutrients, probably due to its fast tillering, initial shoot and root growth rates and its high stock density (Bertholdsson, 2005; Isensee et al., 1994; Seavers & Wright, 1999). The miscanthus proportion in fertilized barley was 3.4% and, as mentioned above, it had the lowest amount of miscanthus biomass of all crops. Therefore, we conclude that barley was the most effective of all tested crops at suppressing resprouting miscanthus after a removal.

However, at 2.65 t DM/ha (+N) and 0.72 t DM/ha (−N), the yield of barley grain in our study was low compared, for example, to an average yield of 7.34 t DM/ha for the barley variety 'RGT Planet' at the research station 'Ihinger Hof' in 2017. One reason for the low yield in our field trial was that the developed green ears of some barley plants had fallen onto the soil surface. The ryegrass yield in our study (3.21 t DM/ha; first plus second cut) was also quite low. By contrast, Dufossé et al. (2014) found no difference in wheat grain yield between that grown after miscanthus and a control. As we have no explanation for the 'ear loss' in barley and the low ryegrass yield in our study, further research is required to clarify whether both were caused by the previous miscanthus crop or by other environmental conditions.

It should also be mentioned that maize, barley, rapeseed and winter wheat were sprayed with herbicides (MaisTer Power, Axial, Butisan and Atlantis respectively) effective

against grassy weed species. These herbicides had a potential effect on miscanthus resprouting and formed part of crop management.

The miscanthus stand in our study was 4 years old, which means that it was comparatively young. We assume that young miscanthus rhizomes have a higher resprouting rate than in older stands. However, further research is necessary to analyse how crop age influences resprouting of miscanthus rhizomes after removal. Additionally, it should be clarified whether other genotypes, for example, *Miscanthus x giganteus*, have higher resprouting rates than the *M. sinensis* genotype used in our study, as *M. sinensis* grows in tufts.

In summary, we found that different mechanisms are effective in suppressing resprouting miscanthus. Intensive soil tillage in a late-sown crop such as maize can destroy miscanthus that has already sprouted. Frequent mowing, as conducted in ryegrass, can probably exhaust the regrowth, as suggested by Isensee et al. (1994). A dense plant population, as in barley, can suppress miscanthus through competition for nutrients, light and water. Which of the spring crops is cultivated after miscanthus is each farmer's individual decision and will be linked to the farm's fodder requirements. However, as the yield of the following crop is likely to be a crucial criterion for the farmer, we recommend cultivating maize, due to its high yields and sufficient miscanthus suppression.

4.2 | How to optimize nitrogen management after a miscanthus removal

As mentioned in the introduction, the new German fertiliser ordinance requires the possible N provision from previous crops to be considered for the cultivation of follow-on ones. However, it is not clear whether soil N increases after a miscanthus removal, as with grassland removal, or whether a high C:N ratio leads to a N deficiency, as is sometimes the case after cereal cultivation, impairing a follow-on crop.

The results of our study show that soil mineralized nitrogen (N_{min}) content increased significantly from the first to the second soil sampling in unfertilized as well as fertilized plots at all tested soil depths (Table 5). For example, the total soil N_{min} content of (unfertilized) fallow land increased from 2 kg/ha (after miscanthus harvest; 20 February 2017) to 38.71 kg/ha (before wheat sowing; 16 October 2017) (Table 6). According to Dufossé et al. (2014), the increase in N_{min} content can be attributed to the miscanthus residues, such as litter, roots and rhizomes, remaining on the field. In our study, the amounts were approximately 1.03 t DM/ha (N content about 12 kg/ha) for litter and 4.77 t DM/ha (N content about 27 kg/ha) for roots and rhizomes (results not shown). Dufossé et al. (2014) found a steady increase in N_{min} content from January to March 2012 (miscanthus removal was conducted in three steps between June and September 2011), probably due to increasing temperatures, and reached a peak of 200 kg N_{min}/ha in April/May 2012. According to these findings, the increasing N content of unfertilized plots in our study can be attributed to the miscanthus residues, which were most likely mineralized over the vegetation period of the follow-on spring crops, increasing the soil N_{min} content.

However, the increase in soil N_{min} from d1 to d2 remained at a low level, for example, +13.11 kg/ha in ryegrass +N and +7.57 kg/ha in barley +N, although these were fertilized with 100 kg N and 70 kg N respectively. The N removal through the biomass of these two crops averaged 49 kg N/ha. Thus, the question arises as to what happened to the remaining N applied to ryegrass +N (about 35 kg N/ha) and barley +N (10 kg N/ha). We assume that the additional fertilizer led to a lower soil N_{min} content and attribute this to the so-called 'negative priming effect': the application of mineral fertilizer led to an N immobilization, making less N (temporarily) available to the crop (Kuzyakov, Friedel, & Stahr, 2000). As this 'negative priming effect' was found mainly in ryegrass and barley, the temporary lack of available N could be an explanation for their low yields. This assumption is strengthened by the fact that the N content of ryegrass +N was lower than ryegrass −N, but needs to be confirmed by further studies. If these results are confirmed, ryegrass and barley cannot be recommended to be grown after a miscanthus removal, due to their low yields.

Maize +N, which received the highest amount of fertilizer of all crops (240 kg N/ha), had a removal of 248 kg N/ha through its biomass. On the second sampling date, 24 kg N_{min}/ha were found in the soil, which means that the maize biomass had taken up almost the complete amount of fertilized N applied.

As stated above, a total of 39 kg N/ha were present on the field from litter and rhizomes. However, the average (of all crops and fertilization levels) increase in soil N_{min} was only 14.85 kg N/ha, leading to the conclusion that some N from miscanthus residues was not plant-available and probably fixed in the organic matter, as suggested by Dufossé et al. (2014). It is possible that a large proportion of the residues may have started to decompose but had not completely decomposed in the first year after the miscanthus removal. After the winter wheat was sown, the N_{min} contents stayed comparatively stable over winter in all plots (in soil depth 0–30 cm; results not shown). It then increased again in spring after the winter wheat was fertilized (results not shown). This indicates that even in the second year after a miscanthus removal, a flush of N is not to be expected. For final clarification, further research needs to analyse the decomposition rate of miscanthus residues after its removal under different soil and climatic conditions. In addition, cultivating crops that take up a high amount of N is recommended after a miscanthus removal to avoid losses through N release from decomposing miscanthus residues.

To summarize, our results show that soil N_{min} content increases after a miscanthus removal, but on a low level. It seems that the N from plant residues is partly fixed in organic matter and is thus not plant-available. However, as some N is supplied by the decomposition of miscanthus residues, our findings suggest that the crop cultivated after a miscanthus removal requires less fertilization. In addition, the results revealed that the fertilized crops were more efficient at suppressing resprouting miscanthus and had a higher yield, indicating that fertilization should not be omitted after a miscanthus removal.

Our results point to a 'negative priming effect' in ryegrass +N and barley +N, which probably led to a (temporary) N immobilization. Thus, it is questionable whether these two crops are suitable for cultivation after a miscanthus removal. Maize, by contrast, tolerated the existing soil conditions quite well and seems to be a suitable crop for cultivation after miscanthus.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Table S1: Average air temperature (measured 2 m above soil surface) and precipitation during field trial (February 2017 – July 2018).

	Average air temperature [°C]		**Precipitation [mm]**	
	2017	2018	2017	2018
January	-	3.91	-	89.0
February	3.30	-2.39	40.8	19.4
March	7.15	2.91	63.2	21.2
April	7.12	12.44	29.0	17.4
May	13.62	14.88	47.0	75.1
June	18.34	17.40	72.2	32.5
July	18.24	19.89	109.9	32.0
August	18.05	-	69.3	-
September	11.81	-	52.2	-
October	10.31	-	51.1	-
November	4.00	-	63.0	-
December	1.10	-	32.5	-
Total	-	-	630.2	286.6

4 Miscanthus for biogas production: The effect of genotype, harvest date and ensiling on digestibility and methane hectare yield of miscanthus

4.1 Harvest date and leaf:stem ratio determine methane hectare yield of miscanthus biomass

This sub-chapter investigated the effects of different harvest dates and genotypes on the substrate specific and methane hectare yield. Over a period of two years, the optimal harvest date and genotype characteristics for miscanthus, used as biogas substrate, were analysed in a field trial and biogas batch tests. For this, four miscanthus genotypes were cut at three different harvest dates in autumn.

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ORIGINAL RESEARCH

WILEY GLOBAL CHANGE BIOLOGY BIOENERGY

Harvest date and leaf:stem ratio determine methane hectare yield of miscanthus biomass

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Abstract

The suitability of miscanthus biomass for anaerobic digestion has already been confirmed by several studies. However, it is rarely used as feedstock in biogas plants, mainly due to uncertainty about the optimal harvest regime with regard to the long-term methane hectare yield and resilience of the crop to green cutting. The recommended green-cut date for the only commercially available genotype *Miscanthus* × *giganteus* (*M*×*g*) ranges from September to November. This time-frame is too broad for agricultural practice and needs to be both narrowed down and further specified for different genotypes. The aim of this study was to identify the most suitable harvest window for an autumn green cut of miscanthus, which delivers both a high dry matter and methane yield while securing the long-term productivity of the crop. A further objective was to quantify the effect of genotypic differences, such as leaf to stem ratio, on the substrate-specific biogas and methane yield. For these purposes, a field trial with four genotypes (*M*×*g, GNT1, GNT3, Sin55*) was conducted over 2 years (2016/2017) and harvested at 2-week intervals on three dates between mid-September to mid-October. Methane hectare yield ranged from 3,183 m³ CH_4 ha^{-1} a^{-1} (*Sin55*) to 5,265 m³ CH_4 ha^{-1} a^{-1} (*M*×*g*), which is mainly influenced by dry matter yield. The substrate-specific methane yield was higher for the leaf (311.0 ml CH_4 (g oDM)$^{-1}$) than the stem fraction (285.1 ml CH_4 (g oDM)$^{-1}$) in all genotypes due to lower lignin content of leaves. Of all genotypes, *M*×*g* showed the highest and *Sin55* the lowest nutrient use efficiency. We conclude that miscanthus in Germany should be harvested in October to maximize methane yields and nutrient recycling and minimize yield reduction. Additionally, to increase methane hectare yields even further, future miscanthus breeding should focus on a higher leaf proportion.

KEYWORDS
anaerobic digestion, fibre content, harvest date, methane yield, miscanthus genotypes, nutrient removal

1 | INTRODUCTION

Several studies have shown the suitability of miscanthus biomass for anaerobic digestion (Kiesel & Lewandowski, 2017; Mayer et al., 2014; Wahid et al., 2015). Kiesel and Lewandowski (2017) demonstrated a methane hectare yield potential for *Miscanthus* × *giganteus* of about 6,000 m³

ha^{-1} year^{-1}. This is within the range of maize, the most common biogas crop in Germany, and therefore an important benchmark for all novel biogas crops (FNR, 2017). A similar amount of agricultural land is required for miscanthus as for maize cultivation to supply a biogas plant (Kiesel, Wagner, & Lewandowski, 2017).

Despite these positive results, miscanthus is rarely used as a substrate for anaerobic digestion in Germany. One reason may be the high establishment costs caused by the labour-intensive and expensive rhizome propagation. However, several studies have shown that a decrease in establishment costs can be expected in the years to come through novel establishment techniques such as seed and collar propagation (Clifton-Brown et al., 2017; Mangold, Lewandowski, Xue, & Kiesel, 2017).

Another reason is the uncertainty about the extent to which the necessary green cut in autumn affects the long-term productivity of the crop. Miscanthus is mainly used for combustion and harvested after winter when lignin and dry matter contents are high (Iqbal & Lewandowski, 2014). This harvest date fits in well with the natural growing cycle of the crop, because it allows the relocation of nutrients to the rhizomes and recycling of nutrients via leaf-fall, thus supporting regrowth in the following year (Cadoux, Riche, Yates, & Machet, 2012).

For use in biogas plants, it is necessary to harvest miscanthus green, i.e. before winter. Later harvests are accompanied by an increase in lignin content in the biomass and thus a decrease in digestibility (Fernandes, Bos, Zeeman, Sanders, & van Lier, 2009). A green cut also leads to higher dry matter yields, as harvest occurs before leaf-fall (Kiesel & Lewandowski, 2017). Schmidt, Lemaigre, Ruf, Delfosse, and Emmerling (2017) found significantly higher dry matter yields from a green cut in autumn (up to 27 t ha^{-1}) than from a brown harvest after winter (22 t ha^{-1}).

However, a green cut allows less time for miscanthus to relocate carbohydrates and nutrients to the rhizome, which can have a potentially negative effect on regrowth in the following year. Fritz and Formowitz (2010) and Kiesel and Lewandowski (2017) showed that an early green cut before or in August negatively affects the long-term productivity of miscanthus. Therefore, for Central European climate conditions, a harvest in September to October is recommended, as both high methane hectare yields and a sufficient green-cut tolerance can be achieved (Kiesel & Lewandowski, 2017). Ruf, Schmidt, Delfosse, and Emmerling (2017) found that a green cut in late September negatively affected the yield the following year, but no physiological effects of a green harvest in November. This range of recommended harvest times (September to November) is too broad for agricultural practice and needs to be further refined.

Biomass quality is not only influenced by harvest time but also by physiological properties, for example proportion of leaf and stem biomass. This has already been established for the combustion of miscanthus biomass, with leaves being less suitable than stems due to their higher content of ash and critical elements (Baxter et al., 2014). For anaerobic digestion, it appears to be the reverse. Wahid et al. (2015) investigated six different harvest dates from August to November and found a significantly higher substrate-specific methane yield (SMY) of the leaf fraction than the stem fraction for the genotypes *M.* × *giganteus* and *Miscanthus sinensis* after 31 days of anaerobic digestion. In addition, they found higher specific methane yields and lower cellulose and lignin contents for *M. sinensis* than for *M.* × *giganteus*. However, Wahid et al. (2015) only investigated 1 year and so were unable to assess the effects on the yield the following year, which is crucial for agricultural practice. Furthermore, the effect of a green harvest on yield in the following year and also on the leaf and stem proportions of the biomass has not been sufficiently investigated.

The aim of this study was to identify the most suitable harvest window for an autumn green cut of miscanthus. This harvest window should enable both a high dry matter and methane yield while securing the long-term productivity of the crop. A further objective was to quantify the effect of genotypic differences, such as leaf proportion, on the substrate-specific and methane hectare yield.

For this purpose, a field trial with four different genotypes was conducted over 2 years and harvested three times at 2-week intervals in the period mid-September to mid-October. In addition to the standard cultivar *M.* × *giganteus*, three novel, seed-based hybrids were tested which are assumed to have improved biomass quality for anaerobic digestion. The dry matter yield (DMY), substrate-specific methane yield (SMY), methane hectare yield (MY) and fibre content were determined for the 2 years 2016 and 2017. The leaf and stem proportions were assessed for each genotype, harvest date and year. Additionally, leaf and stem nutrient contents were measured to quantify the nutrient removal of the green cut.

2 | MATERIALS AND METHODS

2.1 | Field trial

The field trial was conducted on a commercially relevant scale at the University of Hohenheim's research station "Unterer Lindenhof" in south-west Germany (48.4° latitude, 9.2° longitude; approximately 480 m a.s.l.). The location is characterized by a long-term average annual air temperature of 6.8°C and an annual precipitation of 942 mm. The soil, which is classified as a stony marl Rendzina, has a high clay and stone content, and tends to be waterlogged. It can thus be considered to be of low

quality. The climate data for the field trial period (2016–2017) are shown in Supporting Information Table S1.

In the field trial, four genotypes, *M.* × *giganteus* (*M*×*g*), *GNT1*, *GNT3* and *Sin55,* were established in strip plots (width:10.5 m × length: 45.5 m) in a randomized complete block design with four replicates for each genotype, except *GNT1*, which only had three field replications, as the establishment of one field plot was not successful. A detailed description of the four genotypes is given in Table 1.

The planting density was two plants/m^2 with a row distance of 75 cm. The trial was not fertilized during the whole experiment. Weed control was performed annually either mechanically or chemically.

Within the strip plots, a smaller "cutting tolerance trial" was established in a randomized split-plot design, where genotype was the main-plot factor and harvest time was the sub-plot factor. This resulted in 45 plots (three genotypes × four plots × three harvest dates + one genotype × three plots × three harvest dates). Each of those 45 harvest plots had a size of approximately 12 m². The cutting tolerance trial started in the second year after establishment of the miscanthus crop and three different harvest dates were tested. The first harvest date was mid-September (HD 1; 21 September 2016; 18 September 2017), the second 2 weeks later at the beginning of October (HD 2; 4 October 2016/2017) and the third mid-October (HD 3, 17 October 2016/2017).

The harvest procedure was the same for each harvest date in both years. First, the front border of each plot was cut and removed, then eight plants were harvested from the centre of the plot with a "Baural" field trial harvester at a cutting height of 20 cm. The exact area harvested was measured to determine the fresh matter yield (FMY)/ha. To identify the dry matter content (DMC) of the chopped material, a subsample of approximately 1 kg was taken from each plot and dried in a drying cabinet at 60°C to constant weight. The dry matter yield (DMY; t/ha) was estimated based on the FMY and the DMC of the subsample.

Ten randomly selected stems were collected from the remaining borders and separated into leaf and stem fractions. In 2016, 20 stems of the genotype *Sin55* were cut, as ten stems would not have given enough plant material for the analysis. The fractions "leaf" and "stem" were also dried at 60°C to constant weight to identify DMC. After the ten stems had been collected, the remaining borders of each harvest plot were also removed.

2.2 | Biogas batch test

To determine the substrate-specific methane yield (SMY) of the leaf and stem fractions, the dried biomass samples were milled using a cutting mill equipped with a 1-mm sieve (SM 200; Retsch GmbH, Haan, Germany). They were then analysed in a biogas batch test according to VDI guideline 4630. A detailed description of this batch test can be found in Kiesel and Lewandowski (2017). Methane hectare yield was calculated by multiplication of substrate-specific methane yield and the organic dry matter yield.

2.3 | Laboratory analysis

For each laboratory analysis, a subsample was taken from the leaf and stem fractions.

The ash content was determined by incinerating all samples in a muffle kiln at 550°C for 4 hr according to VDLUFA book III, method 8.1 (Naumann & Bassler, 1976/2012).

Lignin, cellulose and hemicellulose content of leaf and stem were analysed by near-infrared spectroscopy (NIRS). Validation and calibration samples were analysed with an ANKOM^{2000} Fiber Analyser and Daisy II Incubator (ANKOM Technology, Macedon, USA). The contents of

TABLE 1 Detailed description of the four genotypes used in the field trial

	Miscanthus × *giganteus* (*M*×*g*)	*GNT1*	*GNT3*	*Sin55*
Type of genotype	Natural hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus*	Artificial hybrids of *Miscanthus sinensis* and *Miscanthus sacchariflorus*		*Miscanthus sinensis* genotype
Origin	South-east Asia	Miscanthus breeding programme of Aberystwyth University		
Propagation characteristics	Vegetative propagation via rhizomes or in vitro culture	Seed propagation		
Senescence characteristics	Early senescence	Later senescence than *M*×*g*		Stay-green genotype (delayed senescence compared to *M*×*g, GNT1* and *GNT3*)
Additional information	Currently, single commercially available genotype			High leaf proportion

neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to VDLUFA book III, method 6.5.1 (NDF), 6.5.2 (ADF), and 6.5.3 (ADL), Naumann & Bassler, 1976/2012;. Table 2 shows the standard error of the NIRS calibration (SEC) and prediction (SEP) and the R^2 of the NIRS calibration and validation. The ADL content is given by the lignin content. Hemicellulose content is determined by subtracting ADF from NDF, cellulose by ADL from ADF.

Nitrogen (N) contents of leaf and stem were analysed using a Vario Max CNS (Elementar Analysensysteme GmbH, Langenselbold, Germany), as described in the VDLUFA Method Book III, method 4.1.2 and DIN ISO 5725.

The phosphorus (P) and potassium (K) contents of leaf and stem were analysed according to the VDLUFA Method Book III, method 10.8.1. For this analysis, 0.5 g of each sample was first dissolved in 8 ml HNO_3, 1 ml H_2O and 5 ml H_2O_2 and then placed in an ETHOS.lab microwave (MLS GmbH, Leutkirch, Germany) for pressure and temperature digestion. Potassium and phosphorus contents were measured with an ICP-OES.

2.4 | Statistical analysis

The experiment was performed in two phases. The first phase consisted of a field trial; in the second phase, samples from the field trial were processed in the laboratory.

The SMY of the leaf and stem fraction were each analysed by a linear mixed model, which considered both field trial and laboratory design (Equation 1).

$$y_{hijkl} = \mu + g_i + d_h + f_j + (gd)_{ih} + (df)_{hj} + (gf)_{ij} + (gdf)_{ihj} + s_l + (gs)_{il} + r_k + e_{hijlk} \quad (1)$$

where y_{hijkl} is the measurement of the *i-th* genotype on the *h-th* harvest date with the *j-th* effect of year in the *l-th* field replication and the *k-th* laboratory replicate. μ is the general effect, g_i is the *i-th* genotype effect *(M×g*; *GNT1*; *GNT3*; *Sin55*), d_h is the main effect of the *h-th* harvest date (HD 1; HD 2; HD 3), f_j is the main effect of the *j-th* year (2016; 2017), $(gd)_{ih}$ is the interaction effect of the *i-th* genotype with the *h-th* harvest date, $(df)_{hj}$ is the interaction of the *h-th* harvest date with the *j-th* year, $(gf)_{ij}$ is the interaction of the *i-th* genotype with the *j-th* year, $(gdf)_{hij}$ is the interaction of the *i-th* genotype with the *h-th* harvest date and the *j-th* year. While all effects described above were taken as fixed, the remaining effects were taken as random. s_l is the random effect of the *l-th* replicate in the first phase (field), r_k is the random effect of the *k-th* replicate in the second phase (laboratory) and e_{hijkl} is the residual error term corresponding to y_{hijkl}. Furthermore, $(gs)_{il}$ is the main-plot error effect associated with main plots of genotype *i* in replicate *l*.

All other traits (DMY, MY, fibre and nutrient content) were measured for both leaf and stem after the first phase; thus for these traits the effects of the *k-th* replicate in the laboratory were dropped from the model in Equation 1.

In all analyses, residuals were graphically checked for normality and homogeneity of variance. For phosphorus (P), log transformation of the data was necessary. Where significant differences were found using an F test, a multiple *t* test (LSD) with $\alpha = 0.05$ was performed. All data analysis was performed using the PROC MIXED procedure of Statistical Analysis Software SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

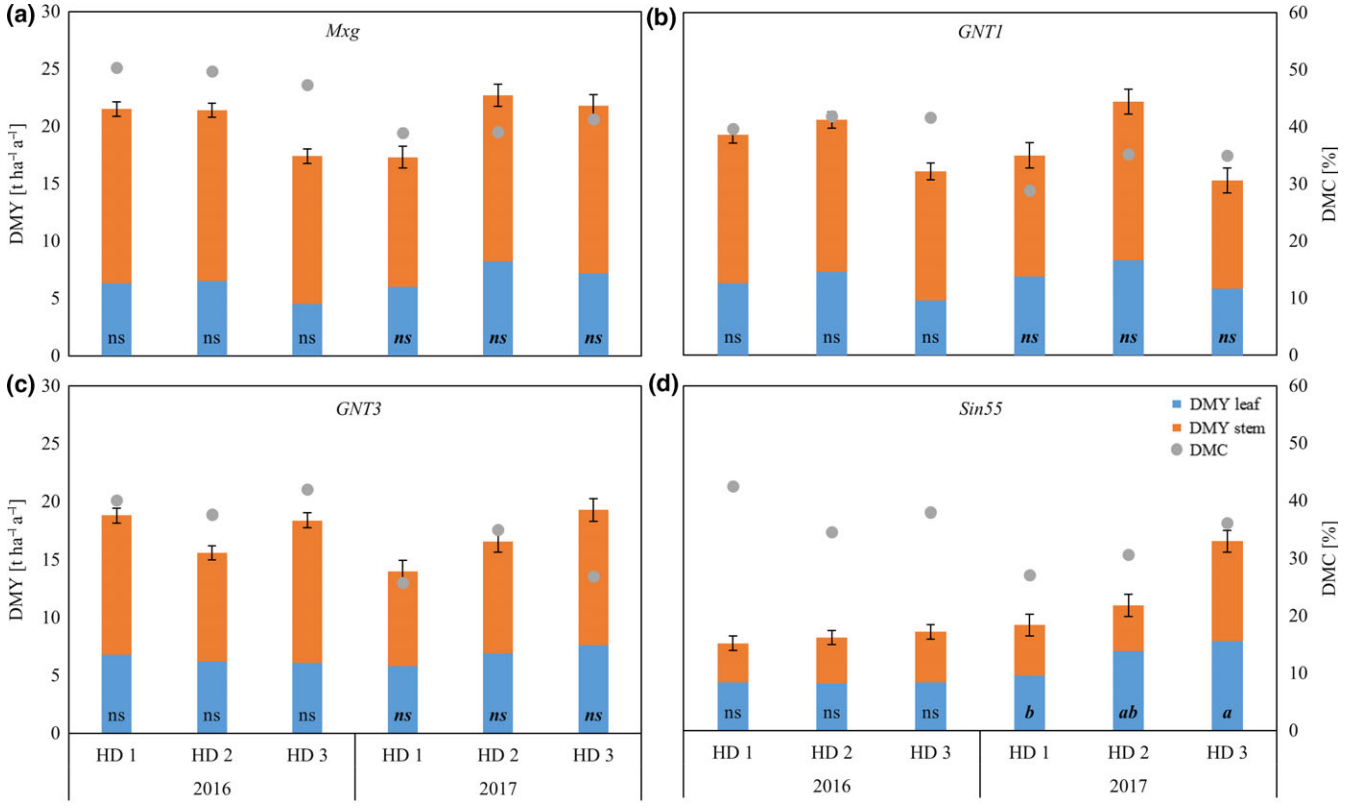
3.1 | Dry matter yield and leaf proportion

Figure 1 shows the DMY and dry matter content (DMC) of the four genotypes on the three harvest dates in 2016 and 2017. The interactions of genotype and year as well as harvest date and year affected dry matter yield of both leaf and stem (Table 3).

Of all four genotypes, *M×g* yielded highest (average: 19.89 t DM ha^{-1} a^{-1}) and *Sin55* lowest (10.83 t DM ha^{-1} a^{-1}) in both years. Both *M×g* and *Sin55* had a higher average yield in 2017 than in 2016; for *GNT1* and *GNT3* it

TABLE 2 NIRS calibration and validation characteristics

	Calibration			Validation		
	Number of samples	Standard error of calibration	R^2	Number of samples	Standard error of prediction	R^2
NDF 2016	181	1.2343	0.9595	25	1.234	0.812
ADF 2016	183	1.3089	0.9615	25	1.271	0.973
ADL 2016	182	0.6764	0.8837	25	0.733	0.887
NDF 2017	195	1.1555	0.9637	45	2.248	0.82
ADF 2017	194	1.1693	0.9695	45	3.77	0.802
ADL 2017	195	0.7153	0.837	45	3.34	0.019



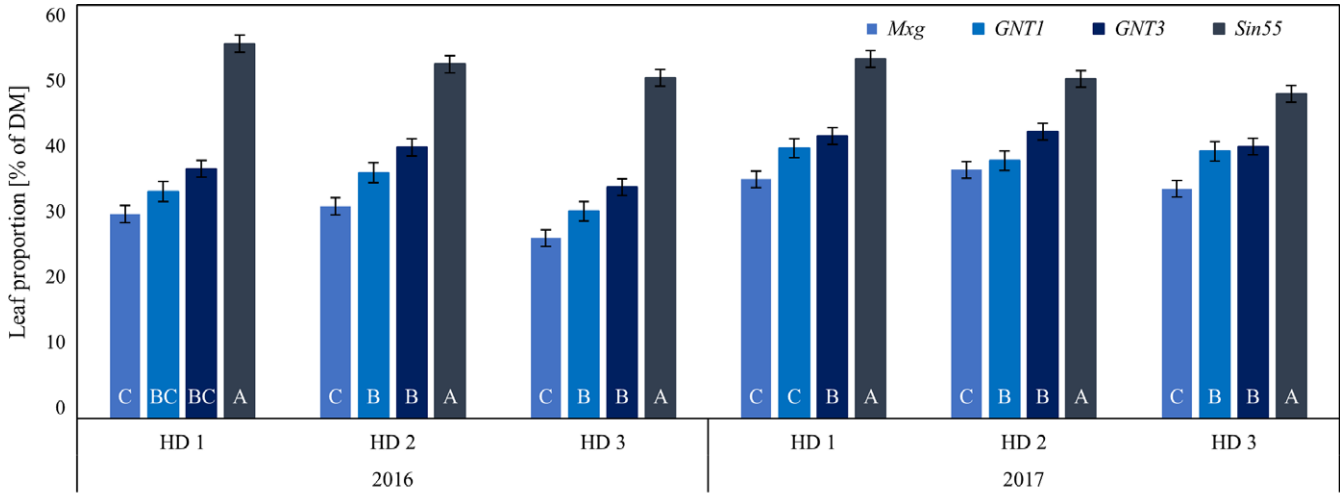
	HD 1		HD 2		HD 3	
	2016	2017	2016	2017	2016	2017
Mxg	A	B	**B**	**A**	*B*	*A*
GNT1	NS	NS	**NS**	**NS**	*NS*	*NS*
GNT3	A	B	**NS**	**NS**	*NS*	*NS*
Sin55	NS	NS	**B**	**A**	*B*	*A*

FIGURE 1 Average dry matter yield (DMY; bars; t ha^{-1} a^{-1}) and dry matter content (DMC; dots; %) of the four genotypes (*M*×*g* [a], *GNT1* [b], *GNT3* [c], and *Sin55* [d]) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September, HD 2: beginning of October, HD 3: mid-October). The blue section of bars represents leaf proportion, the orange section stem proportion. Different lower-case letters within the bars represent significant differences for DMY between harvest dates for each year and genotype (ns: not significant; standard letters for 2016; bold italic letters for 2017). The lower table presents significant differences between the years (2016/2017) for each harvest date and genotype. Standard letters indicate differences for HD 1, bold letters for HD 2 and italic letters for HD 3. Means with same letters were not significant different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard error for DMY

TABLE 3 *p*-values for F tests of fixed effects ($\alpha = 0.05$) for dry matter yield (DMY), substrate specific methane yield (SMY), methane hectare yield (MY) (leaf and stem), and the leaf proportion (leaf prop; total crop)

Source	DMY_{leaf}	DMY_{stem}	SMY_{leaf}	SMY_{stem}	MY_{leaf}	MY_{stem}	Leaf prop
Genotype (Geno)	0.0836	<0.0001	<0.0001	<0.0001	<0.0001	0.1273	<0.0001
Harvest date (HD)	0.1435	0.3244	0.0510	0.1449	0.3953	0.0388	0.0002
Year	<0.0001	0.3602	0.8126	0.0048	0.0017	<0.0001	0.0017
HD × year	0.0026	0.0040	0.1625	0.0007	0.0117	0.0759	0.0274
Geno × HD	0.2849	0.2442	0.8218	0.2409	0.1959	0.3869	0.1701
Geno × year	0.00520	<0.0001	0.0829	0.0421	<0.0001	0.0038	0.0058
Geno × HD × year	0.8527	0.6171	0.3387	0.0170	0.2680	0.4184	0.4006

was the reverse. For the genotypes *M*×*g*, *GNT1* and *GNT3*, the differences in DMY between the three harvest dates within a year were not significant. However, there was a clear trend for all three genotypes that the HD 1 yield was lower, but the HD 2 and 3 yields were similar or slightly higher in 2017 than 2016. Indeed, *Sin55* almost doubled



	HD 1	HD 2	HD 3	HD 1	HD 2	HD 3
Mxg	a	a	b	**ns**	**ns**	**ns**
GNT1	ab	a	b	**ns**	**ns**	**ns**
GNT3	a	ab	b	**ns**	**ns**	**ns**
Sin55	a	ab	b	**a**	**ab**	**b**

FIGURE 2 Average leaf proportion of the four genotypes (*M×g*, *GNT1*, *GNT3* and *Sin55*) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September, HD 2: beginning of October, HD 3: mid-October). The white letters within the bars indicate significant differences in leaf proportion between the genotypes for each harvest date and year. The lower table shows significant differences in leaf proportion between harvest dates for each year (standard letters for 2016, bold letters for 2017) and genotype. Means with same letters were not significant different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard error for leaf proportion

the yield at HD 2 and 3 in 2017 compared with 2016, while at HD 1 yield level was similar for both years. Additionally, *Sin55*, yielded significantly higher at HD 3 than at HD 1 in 2017 (Figure 1d).

On average, *M×g* had the highest (44.3%) and *GNT3* the lowest (34.7%) DMC. The DMC of all genotypes was higher 2016 than in 2017 (Figure 1). The average DMC of all genotypes was highest at HD 1 (43.1%) in 2016. In 2017, the highest DMCs were recorded at HD 2 (35.0%) and HD 3 (34.9%).

Except for the genotype *Sin55*, the dry matter yield was mainly made up of stems. As shown in Figure 2, the average leaf proportion was lowest in genotype *M×g* (31.7% of DM) and highest in *Sin55* (50.6%). All genotypes (except *Sin55*) had a higher leaf proportion in 2017 than 2016. The leaf proportion was lowest at HD 3 for all genotypes in both years.

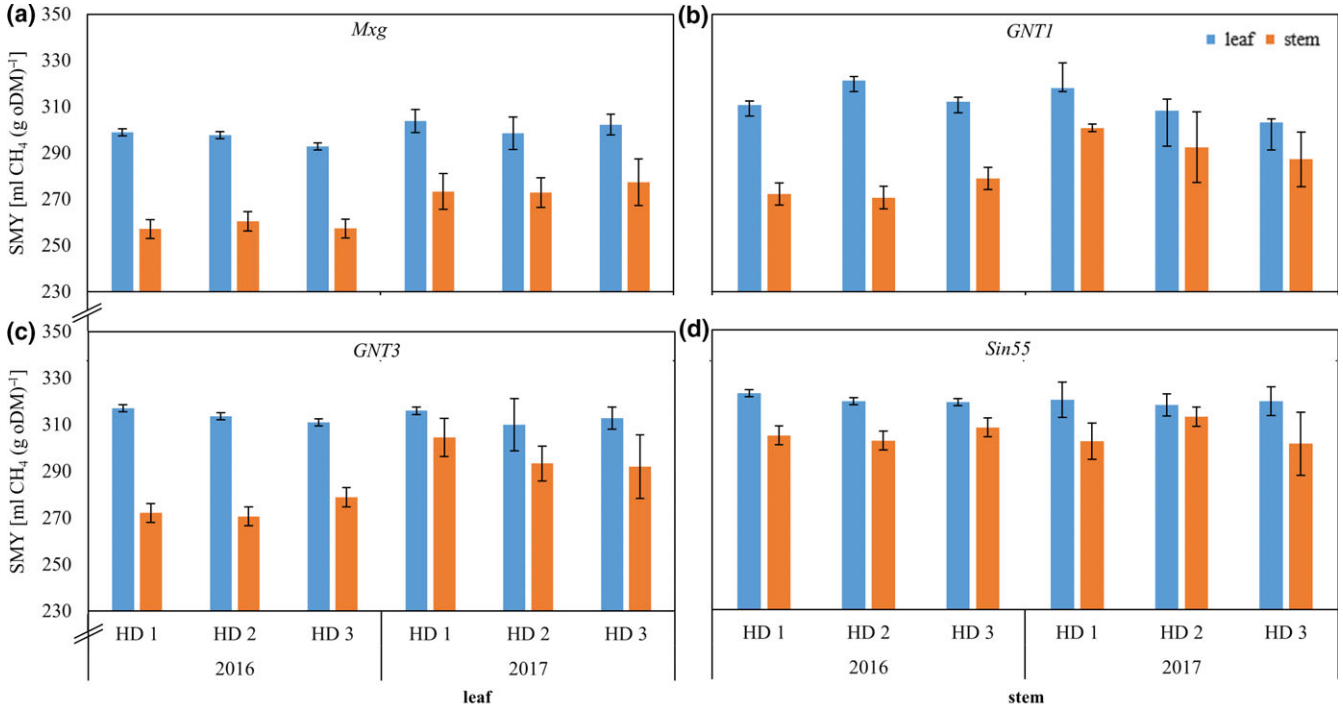
3.2 | Methane yield

The substrate-specific methane yield (SMY) of both leaf and stem biomass was significantly affected by genotype, but not by harvest date (Table 3). The average SMY of all genotypes was 311.0 ml CH_4 (g oDM)$^{-1}$ for the leaf fraction and 285.1 ml CH_4 (g oDM)$^{-1}$ for the stem fraction (Figure 3).

The highest SMY (average of all three HDs) was found in *Sin55* (Figure 3d; leaf: 319.6 ml CH_4 (g oDM)$^{-1}$; stem: 305.1 ml CH_4 (g oDM)$^{-1}$); the lowest was found in *M×g* (leaf: 299.0 ml CH_4 (g oDM)$^{-1}$; stem: 266.3 ml CH_4 (g oDM)$^{-1}$; Figure 3a).

A comparison of the three HDs of each year revealed no clear trends in substrate-specific methane yield. On average, *M×g*, *GNT1* and *GNT3* had higher SMY in 2017 than 2016, while that of *Sin55* was similar in both years.

The methane hectare yield (MY) of leaf and stem fractions was significantly affected by year and genotype × year (Table 3). As shown in Figure 4, average methane hectare yield (MY) was highest for *M×g* (5,265 m³ CH_4 ha^{-1} a^{-1}) and lowest for *Sin55* (3,183 m³ CH_4 ha^{-1} a^{-1}). In all genotypes, the MY was much more strongly influenced by DMY than by SMY. The contribution of the stem fraction to the MY was higher than that of the leaf fraction in all genotypes, except *Sin55*, where the leaf proportion of the DMY was similar to or higher than the stem proportion. The MY of HD 2 and HD 3 was higher in 2017 than 2016 for all genotypes (except *GNT1* at HD 3). The MY of HD 1 was lower in 2017 than 2016 in all genotypes except *Sin55,* where it increased slightly from 2016 to 2017. In 2017, *M×g* and *GNT1* had the highest MY at HD 2, *GNT3* and *Sin55* at HD 3 (significant differences only in *Sin55*).



	HD 1 2016	HD 2 2016	HD 3 2016	HD 1 2017	HD 2 2017	HD 3 2017	HD 1 2016	HD 2 2016	HD 3 2016	HD 1 2017	HD 2 2017	HD 3 2017
Mxg	a	a	b	**ns**	**ns**	**ns**	NS	NS	NS	**NS**	**NS**	**NS**
GNT1	b	a	b	**ns**	**ns**	**ns**	NS	NS	NS	**A**	**AB**	**B**
GNT3	a	ab	b	**ns**	**ns**	**ns**	B	B	A	**A**	**B**	**B**
Sin55	ns	ns	ns	**ns**	**ns**	**ns**	AB	B	A	**A**	**AB**	**B**

FIGURE 3 Average substrate-specific methane yield (ml CH_4/[g oDM]) of leaf (blue bars) and stem (orange bars) biomass of the four genotypes (*M×g* [a], *GNT1* [b], *GNT3* [c], and *Sin55* [d]) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September, HD 2: beginning of October, HD 3: mid-October). The lower table shows significant differences between harvest dates for each year and genotype (2016: standard letters; 2017: bold letters; leaf: lower-case letters; stem: upper-case letters, $\alpha = 0.05$). Means with same letters were not significant different from each other. Error bars represent standard error for SMY

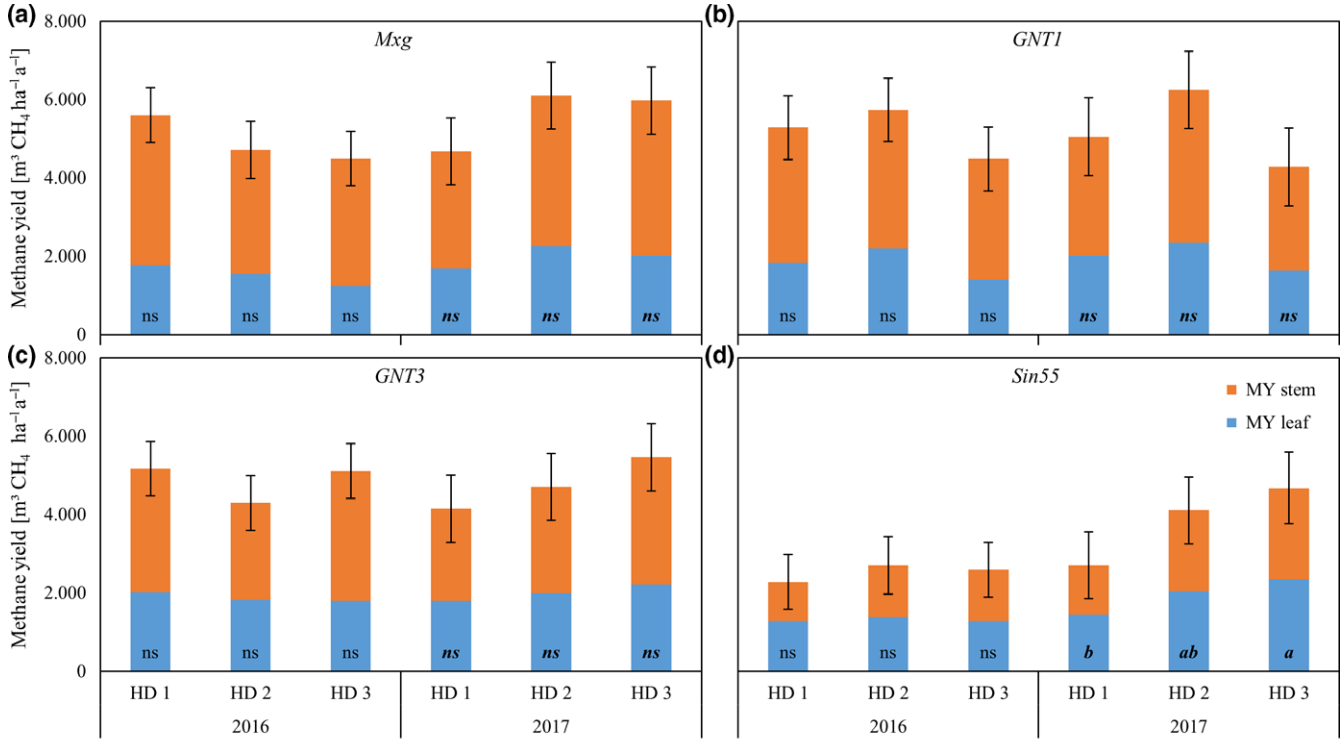
3.3 | Fibre and ash content

The *p*-values for F tests of fixed effects show that all fibre contents, except lignin of leaf fraction, were significantly affected by the interaction of harvest date × year and either genotype or genotype × year (Table 4).

Table 5 shows the fibre (lignin, hemicellulose, cellulose) and ash contents of the four genotypes on the three harvest dates in 2016 and 2017. Genotype *M×g* had the highest average lignin (9.8%) and cellulose (39.2%) content of all genotypes; *GNT1* had the lowest lignin (7.6%) and *Sin55* the lowest cellulose (36.6%) content. However, *Sin55* had the highest (average: 29.7%) hemicellulose content for leaf and stem fraction in both years. *M×g* had the lowest hemicellulose content (average: 25.4%) in both years, except for stem in 2016. Ash content was lowest for *M×g* (5.0%) and highest for *GNT1* (6.1%). For all genotypes and harvest dates in both years, the lignin and cellulose contents were higher in the stem fraction than the leaf fraction (except *GNT1* and *Sin55* at HD 1 in 2017). For hemicellulose and ash contents, it was the reverse (Table 5).

The nutrient removal was significantly affected by the interaction of genotype and year, except for K_{stem} (Table 4). The average removal over all genotypes, harvest dates and years was 115 kg ha^{-1} a^{-1} for nitrogen (N), 257 kg ha^{-1} a^{-1} for potassium (K) and 17 kg ha^{-1} a^{-1} for phosphorus (P). As shown in Figure 5, *GNT1* had the highest nutrient removal of all genotypes (141 kg ha^{-1} a^{-1} N; 301 kg ha^{-1} a^{-1} K; 23 kg ha^{-1} a^{-1} P), *Sin55* the lowest of N (81 kg ha^{-1} a^{-1}) and K (193 kg ha^{-1} a^{-1}), and *M×g* the lowest of P (11 kg ha^{-1} a^{-1}). The average removal of all three nutrients was higher in 2017 than 2016. In all genotypes, N removal was higher by leaves than stems, for K it was higher in stems than leaves, and for P it was balanced between the two fractions.

A comparison of nutrient use efficiency (NUE; biomass produced in kg per nutrient removal in kg) shows that, on average, *M×g* produced the most biomass per removed



	HD 1		HD 2		HD 3	
	2016	2017	2016	2017	2016	2017
Mxg	A	B	**B**	**A**	*B*	*A*
GNT1	NS	NS	**NS**	**NS**	*NS*	*NS*
GNT3	A	B	**NS**	**NS**	*NS*	*NS*
Sin55	NS	NS	**B**	**A**	*B*	*A*

FIGURE 4 Average methane hectare yield (MY; m³ CH_4 ha^{-1} a^{-1}) of the four genotypes (*M×g* [a], *GNT1* [b], *GNT3* [c], *Sin55* [d]) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September, HD 2: beginning of October, HD 3: mid-October). The blue section of bars represents leaf proportion, the orange section stem proportion. Different lower-case letters within the bars represent significant differences for MY between harvest dates for each year and genotype (ns: not significant; standard letters for 2016; bold italic letters for 2017). The lower table presents significant differences between the years (2016/2017) for each harvest date and genotype. Standard letters indicate differences for HD 1, bold letters for HD 2 and italic letters for HD 3. Means with same letters were not significant different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard error for MY

nitrogen (192.5 kg/kg N), potassium (83.3 kg/kg K) and phosphorus (1878.3 kg/kg P) of all genotypes (results not shown). By contrast, *Sin55*, produced the least biomass per removed nutrient (127.5 kg/kg N; 54.1 kg/kg K); 785.7 kg/kg P). Taken as an average of all genotypes for both years, the NUE was highest at HD 3 for all three nutrients and lowest at HD 1 for K and P and at HD 2 for N (results not shown).

4 | DISCUSSION

The objective of the study was to determine more precisely the optimal harvest date of a green cut of miscanthus, which not only delivers high dry matter and methane yields but also guarantees the long-term productivity of the crop. A further objective was to identify the effects of genotypic differences, such as leaf proportion, on methane hectare yield. The results showed that, for all genotypes, a harvest in October 2016 had no negative effects on the dry matter and methane hectare yields in the following year. The methane hectare yield (MY) was found to be mainly influenced by the stem fraction, due to the higher dry matter yield (DMY) of stems than leaves, except for *Sin55*, which had balanced leaf and stem proportions. Additionally, we determined that the substrate-specific methane yield (SMY) was significantly higher for leaves than for stems in all genotypes.

The following sections discuss these results with a view to identifying the optimal harvest time for miscanthus for utilization in biogas plants. In addition, the genotypic differences of the four genotypes used are analysed to develop recommendations for future miscanthus breeding.

TABLE 4 *p*-values for F tests of fixed effects ($\alpha = 0.05$) of fibre content (cellulose, hemicellulose, lignin), nutrient removal (N, K, P) and ash content

Source	$Cell_{leaf}$	$Cell_{stem}$	$Hemicel_{leaf}$	$Hemicel_{stem}$	$Lignin_{leaf}$	$Lignin_{stem}$	N_{leaf}	N_{stem}	K_{leaf}	K_{stem}	P_{leaf}	P_{stem}	Ash_{leaf}	Ash_{stem}
Genotype (Geno)	0.1740	0.0016	0.0406	<0.0001	0.0030	<0.0001	0.0106	<0.0001	0.4779	<0.0001	0.0032	<0.0001	0.0004	<0.0001
Harvest date (HD)	0.0100	0.0877	0.0049	0.0923	0.0104	0.6099	0.1859	0.6486	0.2928	0.8518	0.0010	0.7344	0.186	0.0088
Year	0.0333	0.0245	<0.0001	0.1813	0.0015	0.4644	<0.0001	0.0226	<0.0001	<0.0001	<0.0001	0.2670	0.0002	<0.0001
HD × year	0.0008	0.0174	0.0276	0.0253	0.1818	0.0002	0.0192	0.0885	0.0005	0.1123	0.2809	0.2868	0.7894	0.0033
Geno × HD	0.5267	0.0838	0.7554	0.3984	0.4758	0.8184	0.1124	0.0344	0.1671	0.0954	0.0019	0.2669	0.3021	0.9921
Geno × year	0.1535	0.8551	<0.0001	0.4388	0.5742	0.8076	<0.0001	<0.0001	<0.0001	0.1087	<0.0001	0.0218	0.2214	0.0008
Geno × HD × year	0.1081	0.0846	0.1182	0.7804	0.4089	0.0391	0.5476	0.1295	0.9073	0.3699	0.0222	0.1629	0.5830	0.8077

4.1 | Optimal harvest date for a green cut of miscanthus

Our study determined that, after 2 years of observation, the optimal harvest date for the genotypes *M×g*, *GNT3* and *Sin55* is mid-October and for *GNT1* the beginning of October. The early cut in mid-September 2016 had a negative yield effect on all genotypes, with a tendency to lower the biomass yield in 2017 at HD 1, except for *Sin55* (Figure 1). The yield was expected to be higher in 2017 than 2016 for all genotypes because 2017 was the third year of the plantation. Although that of *Sin55* was higher in 2017 than 2016, the increase was much lower than expected. It has been observed that yields of miscanthus plantations increase steadily from the establishment year to the 3rd or even 5th year, while the stocks are expanding (Iqbal, Gauder, Claupein, Graeff-Hönninger, & Lewandowski, 2015; Lewandowski, Clifton-Brown, Scurlock, & Huisman, 2000). For this reason, it is not clear how much the yields in this study were determined by plantation age and how much by early harvest regime. However, because the genotypes *M×g* and *GNT3* yielded significantly lower at HD 1 in 2017 than HD 1 in 2016, a harvest of these genotypes in mid-September cannot be recommended. This is in line with the results of Kiesel and Lewandowski (2017) who found the best green-cut tolerance for *M×g* when harvested in mid-October. Schmidt et al. (2017) found a slight decrease in DMY in the second year of green cutting when *M×g* was harvested in September in an older (19 years) stand, but not in a younger (6 years) one. Thus, the long-term effect of an ongoing autumn harvest in a growing miscanthus stand needs to be further investigated, as the 2-year analysis of our study is too short to reach a final conclusion on the best harvest date of miscanthus with its lifetime of up to 20 years. We presume that the later harvest in mid-October provides more time for nutrient relocation and enhances the long lifetime of the stand. Evidence for this can be seen in the nutrient use efficiency (NUE), which was on average highest at HD 3 (mid-October) for all genotypes, after nutrient translocation back to rhizomes has begun.

Although the soil conditions were not optimal (as described above), all four genotypes had satisfactory yields (average: 16.6 t DM ha^{-1} a^{-1}).

In general, the average MY for *M×g* found in our study (5,265 m^3 CH_4 ha^{-1} a^{-1}) is within the range found in other studies, namely 5,000–6,000 CH_4 ha^{-1} a^{-1} (Kiesel & Lewandowski, 2017; Kiesel et al., 2017; Mayer et al., 2014). This is at the lower end of the MY of maize (5,000–7,000 m^3 CH_4 ha^{-1} a^{-1}), as reported by Mayer et al. (2014) and Kiesel et al. (2017). However, it should be noted that MY is mainly influenced by dry matter yield. A comparison of miscanthus and maize grown at the same

TABLE 5 Fibre (lignin, cellulose, hemicellulose) and ash content [% of DM] of the four genotypes on three harvest dates. Significant differences between harvest dates are shown by different lower-case letters (leaf) and upper-case letters (stem), for each genotype for 2016 (standard letters) and 2017 (bold) ($\alpha = 0.05$; ns = not significant). Means with same letters were not significant different from each other

	HD 1 2016		HD 2 2016		HD 3 2016		HD 1 2017		HD 2 2017		HD 3 2017	
Content (%)	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**
M×g												
Lignin	7.8^{a}	12.0^{NS}	7.3^{a}	9.54^{NS}	6.5^{b}	9.5^{NS}	$\mathbf{10.8^{ns}}$	$\mathbf{12.0^{NS}}$	$\mathbf{9.5^{ns}}$	$\mathbf{11.6^{NS}}$	$\mathbf{9.5^{ns}}$	$\mathbf{12.1^{NS}}$
Cellulose	33.2^{b}	44.3^{AB}	34.1^{b}	42.7^{B}	35.7^{a}	46.1^{A}	$\mathbf{29.0^{ns}}$	$\mathbf{47.6^{NS}}$	$\mathbf{31.3^{ns}}$	$\mathbf{47.5^{NS}}$	$\mathbf{31.3^{ns}}$	$\mathbf{47.0^{NS}}$
Hemicell.	31.8^{a}	19.8^{NS}	31.1^{a}	19.7^{NS}	29.4^{b}	19.9^{NS}	$\mathbf{32.1^{a}}$	$\mathbf{20.2^{NS}}$	$\mathbf{30.6^{b}}$	$\mathbf{19.3^{NS}}$	$\mathbf{31.5^{a}}$	$\mathbf{19.4^{NS}}$
Ash	6.9^{ns}	2.6^{NS}	6.8^{ns}	2.4^{NS}	6.5^{ns}	2.5^{NS}	$\mathbf{7.19^{ab}}$	$\mathbf{3.6^{NS}}$	$\mathbf{7.77^{a}}$	$\mathbf{3.1^{NS}}$	$\mathbf{7.16^{b}}$	$\mathbf{3.1^{NS}}$
GNT1												
Lignin	5.9^{ns}	9.6^{NS}	5.2^{ns}	9.2^{NS}	5.3^{ns}	8.8^{NS}	$\mathbf{5.8^{ns}}$	$\mathbf{9.2^{NS}}$	$\mathbf{6.9^{ns}}$	$\mathbf{9.4^{NS}}$	$\mathbf{6.1^{ns}}$	$\mathbf{9.9^{NS}}$
Cellulose	33.9^{ns}	43.9^{A}	33.3^{ns}	42.9^{AB}	32.9^{ns}	40.3^{B}	$\mathbf{32.7^{ns}}$	$\mathbf{44.1^{NS}}$	$\mathbf{32.7^{ns}}$	$\mathbf{45.4^{NS}}$	$\mathbf{33.3^{ns}}$	$\mathbf{44.6^{NS}}$
Hemicell.	32.5^{ns}	20.2^{NS}	33.3^{ns}	19.3^{NS}	32.1^{ns}	19.4^{NS}	$\mathbf{32.7^{ns}}$	$\mathbf{23.1^{NS}}$	$\mathbf{31.6^{ns}}$	$\mathbf{24.6^{NS}}$	$\mathbf{31.9^{ns}}$	$\mathbf{23.8^{NS}}$
Ash	7.2^{ns}	3.3^{NS}	7.6^{ns}	3.4^{NS}	7.2^{ns}	3.4^{NS}	$\mathbf{8.2^{ns}}$	$\mathbf{6.0^{NS}}$	$\mathbf{8.1^{ns}}$	$\mathbf{5.2^{NS}}$	$\mathbf{8.1^{ns}}$	$\mathbf{5.0^{NS}}$
GNT3												
Lignin	6.5^{a}	9.9^{NS}	5.9^{b}	9.8^{NS}	5.7^{b}	9.0^{NS}	$\mathbf{10.2^{ns}}$	$\mathbf{8.7^{B}}$	$\mathbf{6.2^{ns}}$	$\mathbf{9.5^{A}}$	$\mathbf{6.3^{ns}}$	$\mathbf{9.7^{A}}$
Cellulose	34.9^{ns}	43.0^{A}	34.1^{ns}	42.0^{AB}	34.4^{ns}	40.3^{B}	$\mathbf{29.4^{b}}$	$\mathbf{43.0^{NS}}$	$\mathbf{35.2^{ns}}$	$\mathbf{44.6^{NS}}$	$\mathbf{34.4^{ns}}$	$\mathbf{43.1^{NS}}$
Hemicell.	32.1^{ns}	21.3^{B}	32.3^{ns}	23.0^{A}	31.7^{ns}	22.6^{AB}	$\mathbf{32.0^{ns}}$	$\mathbf{22.6^{NS}}$	$\mathbf{31.8^{ns}}$	$\mathbf{23.4^{NS}}$	$\mathbf{31.4^{ns}}$	$\mathbf{22.8^{NS}}$
Ash	6.6^{ns}	3.7^{NS}	6.1^{ns}	3.6^{NS}	6.0^{ns}	3.6^{NS}	$\mathbf{7.4^{ns}}$	$\mathbf{6.3^{A}}$	$\mathbf{7.0^{ns}}$	$\mathbf{5.1^{B}}$	$\mathbf{7.0^{ns}}$	$\mathbf{5.0^{B}}$
Sin55												
Lignin	6.9^{a}	8.2^{NS}	6.0^{b}	8.1^{NS}	5.6^{b}	7.6^{NS}	$\mathbf{11.9^{a}}$	$\mathbf{7.5^{C}}$	$\mathbf{6.1^{b}}$	$\mathbf{8.3^{B}}$	$\mathbf{7.0^{b}}$	$\mathbf{9.4^{A}}$
Cellulose	32.9^{ns}	40.7^{A}	32.5^{ns}	40.3^{A}	32.1^{ns}	37.7^{B}	$\mathbf{28.2^{b}}$	$\mathbf{40.6^{B}}$	$\mathbf{34.8^{a}}$	$\mathbf{43.0^{A}}$	$\mathbf{33.5^{a}}$	$\mathbf{42.6^{AB}}$
Hemicell.	33.7^{ns}	27.1^{NS}	33.0^{ns}	27.8^{NS}	32.6^{ns}	27.7^{NS}	$\mathbf{32.5^{ns}}$	$\mathbf{26.0^{AB}}$	$\mathbf{32.1^{ns}}$	$\mathbf{26.8^{AB}}$	$\mathbf{31.6^{ns}}$	$\mathbf{25.0^{AB}}$
Ash	5.7^{ns}	4.0^{NS}	6.1^{ns}	3.7^{NS}	5.8^{ns}	4.2^{NS}	$\mathbf{6.8^{ns}}$	$\mathbf{5.9^{A}}$	$\mathbf{6.8^{ns}}$	$\mathbf{5.2^{AB}}$	$\mathbf{6.8^{ns}}$	$\mathbf{4.7^{B}}$

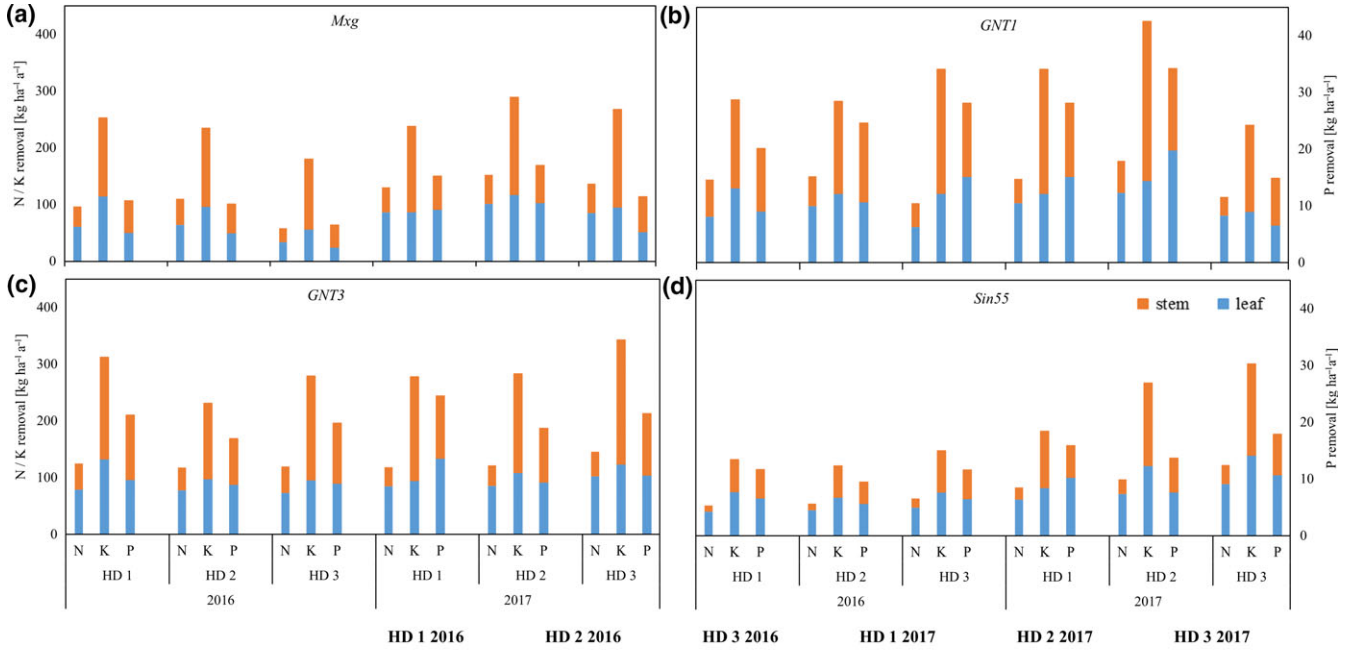
location in 2016/2017 shows that miscanthus would likely have achieved higher MY than maize. This can be deduced from a comparison with maize yields taken from a study by Ehmann, Thumm, and Lewandowski (2018). In this study, the maize plots (which were located adjacent to the field trial in our study) yielded on average approx. 11 t DM ha^{-1} in 2016/2017. A comparison of the DMY for 2016/2017 of this maize with that of the miscanthus in our trial (16.6 t ha^{-1} a^{-1}) reveals a higher DMY for miscanthus.

Additionally, both the production costs and negative environmental impacts are lower for miscanthus than maize biomass under certain conditions, due to lower input requirements for example fertilizer (Wagner et al., 2019). This is mainly a result of the high nutrient use efficiency (NUE) of miscanthus (Cadoux et al., 2012; Lewandowski & Schmidt, 2006). With a view to achieving a high NUE, the latest harvest date in mid-October is preferable. Firstly, the nutrient removal by the biomass is lower. Secondly, the resilience of the crop to a green cut is most likely higher as the nutrients have already partly been relocated from the aboveground biomass to the rhizomes.

As shown by Kiesel and Lewandowski (2017), the nutrient removal of a harvest in mid-October is twice as high as that of a harvest after winter. Thus, to better close nutrient cycles, we recommend the application of digestate in spring to return N, K and P to the field.

When harvested green, miscanthus biomass needs to be ensiled for storage. Therefore, the optimization of the harvest date also has to be considered with regard to the ensiling ability of the biomass. It has been shown that miscanthus biomass is best ensiled when harvested at a dry matter content (DMC) of 35% –40% (Mangold, Lewandowski, Möhring, & Kiesel, 2019). This also indicates that mid-October is the most suitable harvest date.

The lower average DMC found in our study in 2017 can be attributed to the higher accumulated precipitation that year than in 2016. This led to a slower ripening of all genotypes in 2017, resulting in lower average DMCs of 33.3%. However, no recommendations can be provided for optimizing the DMCs through harvest regimes as no clear trends were observed. Generally, it is expected that the DMC increases with senescence and increasing lignin contents of the biomass. Table 5 shows the increase in stem



		HD 1 2016	HD 2 2016	HD 3 2016	HD 1 2017	HD 2 2017	HD 3 2017
Mxg	N	a	a	b	**ns**	**ns**	**ns**
	K	a	ab	b	**ns**	**ns**	**ns**
	P	a	a	b	**ab**	**a**	**b**
GNT1	N	a	a	b	**ns**	**ns**	**ns**
	K	ns	ns	ns	**ab**	**a**	**b**
	P	ns	ns	ns	**a**	**a**	**b**
GNT3	N	ns	ns	ns	**ns**	**ns**	**ns**
	K	a	b	ab	**ns**	**ns**	**ns**
	P	ns	ns	ns	**ns**	**ns**	**ns**
Sin55	N	ns	ns	ns	**ns**	**ns**	**ns**
	K	ns	ns	ns	**ns**	**ns**	**ns**
	P	ns	ns	ns	**ns**	**ns**	**ns**

FIGURE 5 Average nutrient removal (kg ha^{-1} a^{-1}) of the four genotypes (*M×g* [a], *GNT1* [b], *GNT3* [c], *Sin55* [d]) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September; HD 2: beginning of October; HD 3: mid-October). The blue part of the bars indicates the proportion of nutrient removal by the leaves, the orange part the proportion of nutrient removal by stems. The lower table shows significant differences between harvest dates for 2016 (standard letters) and 2017 (bold letters) for each genotype for nitrogen (N), potassium (K) and phosphorus (P). Means with same letters were not significant different from each other. Level of significance was $\alpha = 0.05$

and leaf lignin contents with later harvest for all genotypes. However, the DMC of *GNT3* at HD 3 2017 (shown in Figure 1c) neither followed that trend nor could it be explained by weather conditions. Therefore, further analysis is necessary to clarify the influence of weather conditions, for example on DMC, over a longer time period.

4.2 | Recommendations for miscanthus breeding

Our results demonstrated significant differences in leaf proportion between the four genotypes at all harvest dates in both years (Figure 2). *Sin55* had the significantly highest (50% of DM) leaf proportion, followed by *GNT3* and *GNT1*. *M×g* had the significantly lowest (30% of DM) leaf proportion (Figure 2). A glance at the average SMY of the genotypes reveals a positive correlation between leaf proportion and SMY for all genotypes. This is most likely due to lower lignin contents of leaves than stems (Table 5) as, according to Fernandes et al. (2009), lignin has a low biodegradability. Wahid et al. (2015) also found significantly lower lignin contents of leaves than stems, resulting in a significantly faster biomethane production during the first 31 days of fermentation. Our study found similar results (within the first 10 days) for digestion velocity in 2016 for all genotypes and in 2017 for *M×g* (results not shown). However, as this result could not be confirmed for the other genotypes in 2017, the effect of leaf and stem proportion on digestion velocity needs to be investigated over more years. By contrast, our study confirmed the results of other studies (Kiesel & Lewandowski, 2017; Wahid et al., 2015) that methane hectare yield is mainly

influenced by dry matter yield rather than by SMY. For this reason, miscanthus with a higher leaf proportion in its biomass—and thus higher leaf proportion in its DMY—is more favourable for utilization in anaerobic digestion. In combination with higher SMY, this would result in higher MY and most likely also improve the digestibility. Various studies have revealed a negative correlation between lignin content of biomass and its SMY (von Cossel, Möhring, Kiesel, & Lewandowski, 2017). However, from the results of our study, we cannot conclude that lignin content alone is a suitable criterion for breeding biogas miscanthus. Although leaves had lower lignin contents than stems, leading to higher SMYs, this was not true of the average lignin content of the genotypes. For example, *GNT1* had the lowest lignin content of all genotypes (Table 5) but did not yield the highest average SMY. Additionally, for all genotypes except *GNT1*, the harvest date with the lowest lignin content did not result in the highest SMY. Thus, we cannot recommend selecting miscanthus genotypes for anaerobic digestion on the basis of lignin content alone. Instead, a selection on the basis of hemicellulose content would appear more reasonable as there was a positive correlation between the average hemicellulose content and average SMY in each genotype.

The stay-green genotype *Sin55* was expected to be most suitable for anaerobic digestion on account of late senescence, which leads to lower dry matter contents. It was thus expected to have lower lignin contents, in turn leading to a better digestibility. These expectations, however, were not confirmed by our study. Despite *Sin55* having the highest SMY, it did not have the highest methane hectare yield. This was mainly due to its low DMY, especially in 2016. This leads us to the hypothesis that *Sin55* has a delayed establishment compared with the other three genotypes, resulting in lower yields. We expect that *Sin55* was still in the process of establishing and assume that in 2018 its MY will be similar to that of the other genotypes. In their study, Wahid et al. (2015) demonstrated a higher DMY and biomethane potential, but lower lignin content, for a *M. sinensis* genotype than for *M*×*g*. In addition, they showed that the *M. sinensis* genotype had a slightly higher digestion velocity than *M*×*g* during 35-day fermentation. Our analysis also showed a tendency for higher digestion velocity in *Sin55* than *M*×*g* (results not shown). Better digestibility of the biomass is preferable, as it saves costs for additional pretreatment. Several studies have recommended the pretreatment of miscanthus biomass to gain higher methane yields (Frydendal-Nielsen et al., 2016; Zheng, Zhao, Xu, & Li, 2014), but pretreatment is usually energy- and cost-intensive (Zheng et al., 2014). Therefore, it is likely that, for the genotype *M*×*g*, higher methane yields and thus higher revenues will not necessarily lead to higher profits, as higher costs for pretreatment are incurred. By contrast, *Sin55* could be more profitable, as it requires less pretreatment. Thus, we conclude that stay-green *M. sinensis* genotypes, such as *Sin55*, are most suitable for anaerobic digestion, due to their high SMY and low lignin contents.

In terms of nutrient use efficiency (NUE), *M*×*g* is the most suitable and *Sin55* the least suitable genotype for the supply of low-input biomass for anaerobic digestion, as *M*×*g* produced the most and *Sin55* the least biomass per removed unit of nitrogen, potassium and phosphorus. This is most likely mainly due to the higher yields of *M*×*g* compared with the other genotypes.

In conclusion, this study determined that for the miscanthus genotypes *M*×*g*, *GNT1*, *GNT3* and *Sin55*, the most suitable harvest date for high methane yields is October. A late cut in October is also the most favourable in terms of nutrient use efficiency. In addition, the study found that the leaf fraction of miscanthus biomass produced significantly higher substrate-specific methane yields than the stem fraction, which we attribute to the lower lignin content of leaves. For this reason, future miscanthus breeding should focus on genotypes with a higher leaf proportion (e.g. *M. sinensis* genotype *Sin55*) to increase methane hectare yields.

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SUPPORTING INFORMATION

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Table S1: Average air temperature and precipitation during field trial (2016-2017). Average air temperature was measured 2 m above soil surface.

	Average air temperature [°C]		**Precipitation [mm]**	
	2016	2017	2016	2017
January	2.85	-2.95	68.7	38.6
February	4.17	4.70	70.8	73.3
March	4.19	8.48	40.6	63.7
April	8.26	7.89	76.5	68.2
May	13.20	14.82	91.4	70.4
June	18.21	19.66	119.2	82.3
July	19.46	19.41	50.8	147.8
August	18.78	20.11	37.9	122.0
September	17.36	14.24	34.8	50.9
October	9.04	12.50	49.4	75.5
November	5.08	6.04	65.3	93.8
December	1.66	2.41	3.9	65.3
Sum	-	-	709.3	951.8

4.2 Miscanthus for biogas production: Influence of harvest date and ensiling on digestibility and methane hectare yield

In this sub-chapter, the suitability of miscanthus for ensiling was tested. Parts of miscanthus biomass harvested at three dates and of four different genotypes were ensiled directly after harvesting in WECK-glasses and stored for 90 days. Afterwards, the silage quality was analysed and influence of ensiling on methane yield was investigated in a biogas batch test.

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BIOBASED VALUE CHAINS FOR A GROWING BIOECONOMY

WILEY GLOBAL CHANGE BIOLOGY BIOENERGY

Miscanthus for biogas production: Influence of harvest date and ensiling on digestibility and methane hectare yield

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Abstract

The 8,000 biogas plants currently in operation in Germany are mainly fed with biomass from annual crops. However, feedstock from perennial crops such as miscanthus is expected to be more environmentally benign. If miscanthus is to be used in greater amounts as a substrate for anaerobic digestion, storage will become a relevant topic, as a continuous supply of biomass throughout the year is necessary. The objective of this study was to identify the miscanthus harvest time that best balances the simultaneous achievement of high silage quality, high digestibility and high methane hectare yields. For this purpose, biomass from four miscanthus genotypes with varying senescence characteristics was harvested on three different dates in autumn 2017. Part of the biomass was ensiled, and the methane yield of both ensiled and non-ensiled biomass was analysed in a biogas batch test to assess the effect of ensiling on the methane hectare yield and digestion velocity. The ensiled biomass was found to have an up to 7% higher substrate-specific methane yield and also showed a higher digestion velocity than the non-ensiled biomass. The silage quality was best when miscanthus was harvested in mid-October, due to highest lactic acid content (average: 3.0% of DM) and lowest pH (average: 4.39) compared to the harvests in mid-September and beginning of October. Mass losses during ensiling (as high as 7.6% of fresh matter for the *M. sinensis* genotype *Sin55*) were compensated for by a higher substrate-specific methane yield (up to 353 Nml CH_4 (g oDM)$^{-1}$) in ensiled miscanthus. This resulted in non-significantly different methane hectare yields for non-ensiled (average: 4.635 Nm^3 CH_4/ha) and ensiled miscanthus biomass (4.803 Nm^3 CH_4/ha). A comparison of the four genotypes suggests that *Miscanthus x giganteus* is the most suitable genotype for ensiling as it had the best silage quality.

KEYWORDS

anaerobic digestion, biogas, energy crop, miscanthus genotypes, perennial, silage quality

1 | INTRODUCTION

Currently, there are more than 8,000 biogas plants installed in Germany with an approximate power capacity of 4 GW_{el} (FNR, 2017). The methane produced is most commonly converted into electricity on site. Electricity produced from biogas accounts for approximately 5% of total German electricity generation (FNR, 2017). In future however, the idea is to feed

the methane into the gas grid and use it centrally in larger power stations. The pooling of the produced biomethane via the gas grid has several advantages. If the conversion of methane into electricity and heat takes place at locations with a high heat demand, the overall efficiency and economic viability of biogas plants increases (FNR, 2012; Scholz, Melin, & Wessling, 2013). Moreover, using the gas grid for collection and storage of biomethane facilitates a demand-driven energy supply and is one way in which anaerobic digestion can contribute to balancing out fluctuations in energy supply from wind and photovoltaic (FNR, 2012; Scholz et al., 2013). In addition, biomethane can be used for various other utilization pathways including transportation fuel or chemicals, due to its similarity to natural gas (FNR, 2012; Patrizio, Leduc, Chinese, Dotzauer, & Kraxner, 2015). Today, biogas plants already significantly contribute to the energy mix, but in future are expected to play a crucial role in energy supply systems. This is likely to lead to a stable or even increasing demand for biomass as a substrate for biomethane production.

In Germany, 51% of all biogas plants use biomass crops as feedstock, mostly annual crops (FNR, 2017). Perennial crops such as miscanthus are currently being investigated for their suitability for biogas production (Kiesel, Nunn, & Iqbal, 2017a; Mayer et al., 2014; Ruf, Schmidt, Delfosse, & Emmerling, 2017; Schmidt, Lemaigre, Ruf, Delfosse, & Emmerling, 2018; Wahid et al., 2015). Perennials are expected to be more environmentally benign than annual crops due to their low-input requirements and beneficial environmental profile (Kiesel, Wagner, & Lewandowski, 2017; McCalmont et al., 2017; Wagner et al., 2019). The risk of nutrient leaching and soil erosion, for example, is minimized as a result of undisturbed soil that is covered by vegetation during the whole year (Blanco-Canqui, 2010). In addition, it has been shown that soil organic carbon increases under perennials (Blanco-Canqui, 2010).

If perennials such as miscanthus are to be used in biogas plants with the aim of making biogas production more environmentally benign, several challenges need to be overcome. One of these is to identify the optimal date of a green harvest in autumn. Most studies dealing with green-harvested miscanthus have focused on the question of which harvest date achieves high methane hectare yields, while maintaining the long-term productivity of the crop (Kiesel & Lewandowski, 2017; Schmidt et al., 2018; Wahid et al., 2015). But very few studies have addressed the question of how to store green-harvested miscanthus for anaerobic digestion (Baldini, da Borso, Ferfuia, Zuliani, & Danuso, 2017; Whittaker, Hunt, Misselbrook, & Shield, 2016). Storage, however, is extremely relevant for anaerobic digestion, since a continuous supply of biomass is necessary throughout the year.

Until now, ensiling is the best-known preservation technique for biomass with high water content (Baldini et al., 2017). Whittaker et al. (2016) ensiled *Miscanthus* × *giganteus (M×g)* and *Miscanthus sacchariflorus* harvested in September in Rothamsted (UK) and concluded that additives are necessary. Baldini et al. (2017) demonstrated that *M×g* can be ensiled without additives and showed that, in Italy, the silage quality of miscanthus harvested in autumn was better than that harvested in summer.

Both studies mainly focused on the general feasibility of ensiling miscanthus but did not investigate the extent to which different harvest dates affect the ensiling and subsequent anaerobic digestion. Indeed, the study by Whittaker et al. (2016) found non-ensiled *M×g* to have a non-significant higher biomethane potential than ensiled *M×g*. However, this study only considered one harvest date. Baldini et al. (2017) investigated silage quality from two harvest dates, but did not analyse the effect of ensiled compared to non-ensiled miscanthus on methane hectare yield.

The intention of our study is to investigate both the optimal harvest date of miscanthus and the affects of ensiling on its methane hectare yield. It is known that harvest date of maize effects both processes, ensiling and biogas production (Amon et al., 2007). This is why maize is not harvested when the yield is highest, but when the best silage quality can be expected. However, for miscanthus it is not clear whether the digestibility is affected by the ensiling process. During ensiling, fermentation acids, such as acetic acid, are formed. Acetic acid is an intermediate in the anaerobic digestion process and therefore directly available for methanogen microorganisms. In addition, the fermentation acids may help to reduce the recalcitrance of the lignocellulosic miscanthus biomass and thus positively affect the methane yield of the silage. For this reason, ensiled miscanthus could be expected to be more easily digested than non-ensiled miscanthus. On the other hand, the conversion of sugars into fermentation acids is accompanied by energy losses, which negatively affects methane hectare yields. The question arises whether the two effects compensate each other or whether one is predominant?

The objective of this study was to identify the harvest date that best balances the simultaneous achievement of high silage quality, high digestibility and high methane hectare yield in miscanthus. We hypothesized that a later harvest date would have lower silage quality, lower substrate-specific methane yields and lower digestibility, due to higher dry matter contents. Moreover, we hypothesized that genotypes with earlier senescence would also have lower silage quality and substrate-specific methane yields due to higher dry matter and higher lignin contents. According to Galler (2011), biomass with higher dry matter content builds less lactic acid and is thus more difficult to ensile. In addition, lignin is known to reduce the biodegradability of biomass (von Cossel, Möhring, Kiesel, & Lewandowski, 2018; Fernandes, Bos, Zeeman, Sanders, & van Lier, 2009) and therefore biomass with higher lignin content is expected to have lower methane yields.

TABLE 1 Description of the four genotypes used in the field trial

	Miscanthus × *giganteus (M×g)*	*GNT1*	*GNT3*	*Sin55*
Type of genotype	Natural hybrid of *M. sinensis* and *M. sacchariflorus*	Artificial hybrids of *M. sinensis* and *M. sacchariflorus*		*M. sinensis* genotype
Origin	South-East Asia	Miscanthus breeding programme of Aberystwyth University		
Propagation characteristics	Vegetative propagation via rhizomes or in vitro culture	Seed propagation		
Senescence characteristics	Early senescence	Later senescence than *M×g*		Stay-green genotype (delayed senescence compared to *M×g, GNT1* and *GNT3*)
Additional information	Currently, single commercially available genotype			High leaf proportion

To test our hypotheses, four miscanthus genotypes with varying senescence characteristics were harvested on three different dates in autumn 2017. Part of the biomass was ensiled, and the methane yield of both ensiled and non-ensiled biomass was then analysed in a biogas batch test to assess the effect of ensiling on the methane hectare yield and digestion velocity.

2 | MATERIALS AND METHODS

The experiment was performed in two phases. The first consisted of a field trial; in the second, samples from the field trial were processed in the laboratory.

2.1 | Field trial

Biomass was harvested in 2017 (third growing season) from a field trial at "Unterer Lindenhof," a research station of the University of Hohenheim. The experimental design was a split-plot design with four replications using genotypes as main plot factor and harvest date as sub-plot factor. Detailed information on the field trial is provided in Mangold et al. (2019). An overview of the weather conditions in 2017 can be found in Supporting Information Table S1.

Four different genotypes, *Miscanthus* × *giganteus* (*M×g*), *GNT1*, *GNT3* and *Sin55,* were established, details of which are provided in Table 1. These genotypes were harvested on three different harvest dates (HD) in 2017: mid-September (18 September; HD 1), beginning of October (4 October; HD 2) and mid-October (17 October; HD 3).

At harvest, the border of each plot was removed and eight plants (approx. 4 m^2) were cut at a height of 20 cm using a field trial harvester "Baural." The chopped plant material was weighed and two subsamples of each plot were taken. The harvested area was measured to determine the fresh matter yield (FMY) per hectare. One subsample (subsample 1) was dried in a cabinet dryer at 60°C to constant weight to determine the dry matter content (DMC). The DMY was calculated based on fresh matter yield (FMY) and DMC. The second subsample (subsample 2) was used for the silage trial. The chopped material was filled into plastic bags and transported to the laboratory.

2.2 | Ensiling miscanthus

In the laboratory, subsample 2 was divided into subsample 2a and 2b. Subsample 2a was used to analyse the buffer capacity and methane yield of the raw, non-ensiled biomass. Subsample 2b was ensiled to analyse the silage quality and methane yield. Analyses of silage quality included silage acids (acetic, lactic, propionic, butyric acid), ethanol, sugars and pH.

Subsample 2a was dried at 60°C to constant weight and milled using a cutting mill (SM 200; Retsch GmbH, Haan, Germany) equipped with a 1-mm sieve.

For the analysis of the buffer capacity, a further subsample of 2a was dried again at 105°C for 4 hr in a drying cabinet and sent to an external laboratory (Center for Agricultural Technology [LTZ] Augustenberg, Karlsruhe, Germany). To estimate the buffer capacity, 100 ml distilled water was added to 1 g of the dry samples (ratio 1:100). After 30–60 min,

lactic acid was titrated until a pH value of 4.0 was reached. The buffer capacity was then calculated by Equation 1:

$$BC = (T - BV) \cdot F \cdot \frac{M_{\text{lactic acid}}}{DM} \quad (1)$$

where BC is the buffer capacity, T is the titration value (amount of lactic acid); BV = blind value; F = factor of the 0.1 mol/L lactic acid; $M_{\text{lactic acid}}$ = molecular weight of lactic acid 90.08 g/mol; DM = dry matter content in %.

Subsample 2b was used for the ensiling trial. At each harvest date, the biomass was ensiled a few hours after the harvest. This trial was conducted according to the DLG guideline for the assessment of silage additives (2013). Depending on the DMC of each genotype at harvesting, 550–700 g of the chopped biomass was pressed with a wooden pestle into WECK® glass jars of 1.5-L volume. This resulted in different packing densities: *GNT1*, *GNT3* and *Sin55* had a packing density of 465 kg/m^3 on all three harvest dates, as 700 g of fresh biomass was pressed into each jar. *M*×*g* had a packing density of 400 kg/m^3 on HD 1 and HD 2 (600 g fresh biomass pressed into jars) and a density of 366 kg/m^3 on HD 3 (550 g of fresh biomass). After filling each jar, the rim was cleaned with a paper towel to free it of any biomass particles. The jar was then closed airtight with a rubber ring, a glass lid and two metal clips. This type of sealing ensures that ambient air cannot enter the jar, but that overpressure, originating from gases produced in the ensiling process, is released before critical pressures are reached. Two silage jars were filled from each subsample 2b and thus from each field plot in order to have a "backup" jar should the ensiling of one jar fail. The maximum filling difference was set at 5 g fresh matter over all jars of the same treatment (genotype × harvest date). After all silage jars had been filled, they were stored according to a completely randomized design in a climate chamber (25°C, 60% humidity) for 90 days. The glasses were weighed daily in the first 8 days and then once a week for the remaining storage period to assess the gaseous fresh matter losses of the biomass during the ensiling process.

After the 90-day storage period, the silage jars were removed from the climate chamber and opened. As no fouling or mould was observed in any of the samples, the silage of both jars from the same plot was pooled and a subsample of 50 g taken. This subsample was filled into a plastic bag and stored in a freezer (−20°C) until it was used for silage quality analysis. The remaining silage was dried at 60°C in a drying cabinet to constant weight and then the DMC was calculated. It was then milled following the same protocol as for subsample 2a (cutting mill SM 200 [Retsch GmbH] equipped with a 1-mm sieve). The same procedure was applied for each of the three harvest dates.

Once the ensiling trial from all three harvest dates was complete, the frozen 50-g subsamples of each genotype × harvest date combination were sent to the agricultural centre (LAZBW) Aulendorf for analysis of silage acids (acetic, lactic, propionic and butyric acid), ethanol, sugars and pH.

The silage acids, ethanol and sugars were determined by HPLC analysis. For this, 250 ml distilled water was added to the frozen 50-g subsamples. The water and silage mixture was homogenized twice in a Stomacher 400 circulator on the highest setting, each time for 2 min. An extract was prepared, 10 ml of which was centrifuged for 10 min at 10,000 rpm and then analysed in the HPLC.

2.3 | Biogas batch test

A biogas batch test was conducted according to the VDI guideline 4630 to measure the substrate-specific biogas and methane yield of each "genotype × harvest date combination" for ensiled (subsample 2b after drying and milling as described above) and non-ensiled (subsample 2a after drying and milling) biomass. From each sample, 200 mg oDM (organic dry matter = volatile solids) was filled into a gastight fermentation flask and mixed with 30 g inoculum (4% DM content, 37% ash content). This resulted in an inoculum:substrate ratio of 3.8:1. The inoculum was obtained from the digester of a commercial mesophilic biogas plant that uses maize, grass and cereal whole-crop silage, liquid and solid cattle manure and small quantities of horse manure as substrates. The oDM content was estimated by weight loss during drying of an aliquot of approx. 1 g at 105°C in a cabinet dryer and incineration at 550°C in a muffle kiln to constant weight. The fermentation flasks were placed in a water bath at 39°C in a randomized block design for 35 days. The biogas production was measured via the pressure increase inside the flasks, and the methane content was measured by a GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan). The biogas production was calculated as dry gas under standard conditions (0°C, 1,013 hPa). A detailed description of the biogas batch test method is provided in Kiesel and Lewandowski (2017).

Since both ensiled and non-ensiled samples were analysed, it is important to highlight that all samples were dried at 60°C before analysis in the biogas batch test. Drying silage partly removes volatile organic compounds. For this reason, the DM content of such samples is often corrected. However, in our study, we did not make any corrections to the DM content for two reasons. Analysis of ensiled biomass dried at 60°C gives only minor differences between corrected and uncorrected substrate-specific methane yields (SMY; Mukengele & Oechsner, 2007). In addition, it has been shown by Mukengele and Oechsner (2007) that drying at 60°C almost completely removes acetic acid (93%) and ethanol (98%) (lactic acid was

TABLE 2 *p*-Values for *F* tests of fixed effects ($\alpha = 0.05$) of silage quality

	DMC_{silage}	Lactic acid	Acetic acid	Butyric acid	Ethanol	pH	Fructose	Buffer capacity	Mass losses
Genotype	<0.0001	0.0001	0.0533	0.0713	<0.0001	0.0011	<0.0001	0.0216	0.0019
Harvest date	<0.0001	<0.0001	0.0605	0.0007	0.3683	<0.0001	0.0008	0.0391	<0.0001
Genotype × Harvest date	0.4650	0.3813	0.0873	0.8961	0.6702	0.2787	0.0079	0.1931	0.7407

difficult to measure in their study and therefore volatility rate was only estimated). In our study, the amounts of acetic acid and ethanol present in the silage (0.7% of DM and 0.09% of DM, respectively) were negligible.

Additionally, the digestion velocity of the miscanthus biomass was assessed by the volume of biogas produced per hour. The biogas batch test included an internal laboratory maize standard (harvested in 2012) for comparison purposes and to monitor the biological activity of the inoculum.

The biogas production of each substrate and the velocity (biogas produced per hour) presented in the results section are net values, that is, the biogas production of the inoculum has already been deducted.

The methane hectare yield was calculated by multiplication of substrate-specific methane yield and organic dry matter yield. For the silage treatment, the calculation also took the mass losses during ensiling into account.

2.4 | Statistical analysis

Substrate-specific methane yield (SMY) and methane hectare yield (MY) were analysed by a linear mixed model, which considered both field trial and laboratory design (Equation 2).

$$y_{ihjlk} = \mu + g_i + d_h + f_j + (gd)_{ih} + (df)_{hj} + (gf)_{ij} + (gdf)_{ihj} + s_l + (gs)_{il} + r_k + e_{ihjlk} \quad (2)$$

where y_{ihjlk} is the measurement of the i-th genotype on the h-th harvest date with the j-th effect of ensiling in the l-th field replication and the k-th laboratory replicate. μ is the general effect, g_i is the i-th genotype effect *(M×g*; *GNT1*; *GNT3*; *Sin55*), d_h is the main effect of the h-th harvest date (HD 1; HD 2; HD 3), f_j is the main effect of the j-th ensiling (non-ensiled; ensiled), $(gd)_{ih}$ is the interaction effect of the i-th genotype with the h-th harvest date, $(df)_{hj}$ is the interaction effect of the h-th harvest date with the j-th ensiling, $(gf)_{ij}$ is the interaction effect of the i-th genotype with the j-th ensiling, $(gdf)_{ihj}$ is the interaction effect of the i-th genotype with the h-th harvest date and the j-th ensiling, s_l is the random effect of the l-th replicate in the first phase (field), r_k is the random effect of the k-th replication in the second phase (laboratory), and $(gs)_{il}$ is the main plot error associated with the area where genotype i in replicate l is grown. e_{ihjlk} is the residual error term corresponding to y_{ihjlk}.

As no replicates were performed in the laboratory for the silage quality parameters (silage acids, pH value, sugar content, buffer capacity, mass losses), r_k was removed from the model. In addition, where only ensiled samples were analysed, all effects including ensiling (f_j) were dropped from Equation 2. Thus, the model simplifies to Equation 3.

$$y_{ihl} = \mu + g_i + d_h + (gd)_{ih} + s_l + (gs)_{il} + e_{ihl}, \quad (3)$$

where all effects are denoted as on Equation 2. In all analyses, residuals were graphically checked for normality and homogeneity of variance. Where significant differences were found using an F test, a multiple t test (LSD) with $\alpha = 0.05$ was performed. A letter display using identical letters for means which are not significant from each other was used. Additionally, simple means were calculated for presentation purpose only. All data analysis was performed using the PROC MIXED procedure of Statistical Analysis Software SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Dry matter yield

The highest average dry matter yield (DMY) over all three harvest dates was found in genotype *M×g* (20.69 t DM/ha) and the lowest in *Sin55* (13.26 t DM/ha), with genotypes *GNT1* (18.51 t DM/ha) and *GNT3* (16.69 t DM/ha) in between. The differences in DMY between the three harvest dates were only significant for *Sin55*, which had the highest yield at HD 3 (16.59 t DM/ha) and the lowest at HD 1 (9.29 t DM/ha). Genotype *GNT1* had the lowest yield at HD 3, the other three at HD 1.

The average dry matter content (DMC) of all four genotypes and all harvest dates was 33.4%. *M×g* had the highest DMC (39.7%) and *GNT3* the lowest (29.4%). Detailed results for the dry matter yields and contents are shown in Mangold et al. (2019).

3.2 | Silage quality

The silage quality of the genotypes was assessed by analysing the buffer capacity, the content of a number of silage acids, ethanol and sugars, and the pH of the silage.

The test for fixed effects (Table 2) showed highly significant impacts of harvest date on dry matter content (DMC_{silage}), lactic acid content, pH value and mass losses within a genotype (level of significance $\alpha = 0.05$). DMC_{silage}, lactic acid, ethanol and fructose contents were highly affected by genotype (Table 2). The interactions of genotype × harvest date were only significant for the parameter fructose content. An overview of all results relevant for the silage quality is given in Supporting Information Table S2.

The average dry matter content of the silage was significantly higher for *M×g* (36.7%) compared to 28.6%–30.2% for the other genotypes (see Table 4).

The lactic acid content increased with later harvest date (Table 3). Of all genotypes, *M×g* had the highest lactic acid content (average: 2.97% of DM) and *Sin55* the lowest (average: 0.99% of DM; Table 4). Across genotypes, HD 3 had the significantly highest lactic acid content (Table 3). Acetic acid content was highest in the genotypes *GNT3* and *Sin55* (average content: 0.9% of DM). Butyric acid content was significantly lowest at HD 3 for all genotypes. *M×g* had the lowest average butyric acid content (0.07% of DM) and *Sin55* the highest (0.15% of DM). The propionic acid content was so low in all genotypes that the results are not presented here. The ethanol content of all genotypes was not significantly different between the harvest dates. *M×g*, however, had a significantly higher ethanol content compared to the other genotypes (Table 4).

TABLE 3 Marginal means of silage quality parameters with their standard error for harvest date. For each parameter, means with identical letters are not significantly different from each other

Quality parameter	HD 1	HD 2	HD 3
DMC_{silage} (%)	$29.56^{b} \pm 0.39$	$29.41^{b} \pm 0.39$	$34.68^{a} \pm 0.39$
Lactic acid (% of DM)	$0.66^{b} \pm 0.27$	$1.09^{b} \pm 0.27$	$3.06^{a} \pm 0.27$
Butyric acid (% of DM)	$0.17^{a} \pm 0.02$	$0.16^{a} \pm 0.02$	$0.06^{b} \pm 0.02$
pH	$5.13^{a} \pm 0.07$	$4.97^{a} \pm 0.07$	$4.39^{b} \pm 0.07$
Buffer capacity	$3.67^{b} \pm 0.22$	$4.21^{ab} \pm 0.22$	$4.50^{a} \pm 0.22$
Mass losses (% of FM)	$6.59^{a} \pm 0.37$	$6.56^{a} \pm 0.37$	$4.28^{b} \pm 0.37$

The ensiling process requires a low pH of max. 4.5 to perform sufficiently and ensure stable preservation of the biomass (Galler, 2011). This pH value was achieved by all genotypes on HD 3, by *M×g* even on HD 2 (Supporting Information Table S2). *M×g* had the lowest average pH value (4.5), the other three genotypes had the same average pH value (4.9; Table 4).

Glucose and sucrose were not detectable in the biomass (data not shown); fructose content was low (Supporting Information Table S2). The average buffer capacity was lowest for *M×g* (3.58) and highest for *GNT1* (4.67; Table 4). The mass losses during ensiling decreased significantly with later harvest date in each genotype (Table 3). *M×g* had the lowest average mass losses (4.3% of FM) and *Sin55* the highest (6.4% of FM; Table 4).

3.3 | Substrate-specific and methane hectare yield

As shown in Table 5, the substrate-specific methane yield (SMY) was affected by interactions of harvest date with genotype and ensiling. Figure 1 shows the mean values of harvest date-by-ensiling combinations for the SMY. Taken as an average across all genotypes, the SMY tends to decrease from HD 1 to HD 3 (Figure 1). Additionally, it can be seen that, as an average across all genotypes, non-ensiled

Quality parameter	*M×g*	*GNT1*	*GNT3*	*Sin55*
DMC_{silage} (%)	$36.7^{a} \pm 0.43$	$30.24^{b} \pm 0.52$	$29.28^{bc} \pm 0.43$	$28.66^{c} \pm 0.43$
Lactic acid (% of DM)	$2.97^{a} \pm 0.30$	$1.24^{b} \pm 0.34$	$1.21^{b} \pm 0.30$	$0.99^{b} \pm 0.30$
Ethanol (% of DM)	$0.12^{a} \pm 0.004$	$0.08^{b} \pm 0.005$	$0.09^{b} \pm 0.004$	$0.09^{b} \pm 0.004$
pH	$4.51^{b} \pm 0.08$	$4.97^{a} \pm 0.09$	$4.93^{a} \pm 0.08$	$4.91^{a} \pm 0.08$
Buffer capacity	$3.58^{c} \pm 0.25$	$4.67^{a} \pm 0.29$	$4.42^{ab} \pm 0.25$	$3.83^{bc} \pm 0.27$
Mass losses (% of FM)	$4.30^{b} \pm 0.41$	$6.23^{a} \pm 0.48$	$6.29^{a} \pm 0.41$	$6.43^{a} \pm 0.41$

TABLE 4 Marginal means of silage quality parameters with their standard error for genotype. For each parameter, means with identical letters are not significantly different from each other

biomass had a significantly lower SMY than ensiled biomass at each harvest date (Figure 1). The genotypes *M×g* and *GNT1* had on average 7%, and the genotypes *GNT3* and *Sin55* on average over 6% higher SMY for the ensiled than the non-ensiled biomass.

TABLE 5 *p*-Values for *F* tests of fixed effects ($\alpha = 0.05$) of substrate-specific methane yield (SMY) and methane hectare yield (MY)

	SMY	MY
Genotype	<0.0001	<0.0001
Harvest date	0.0005	0.0032
Ensiling	<0.0001	0.5067
Genotype × Harvest date	0.0212	0.0483
Harvest date × Ensiling	0.0315	0.8620
Genotype × Ensiling	0.3865	0.9447
Genotype × Harvest date × Ensiling	0.1097	0.9995

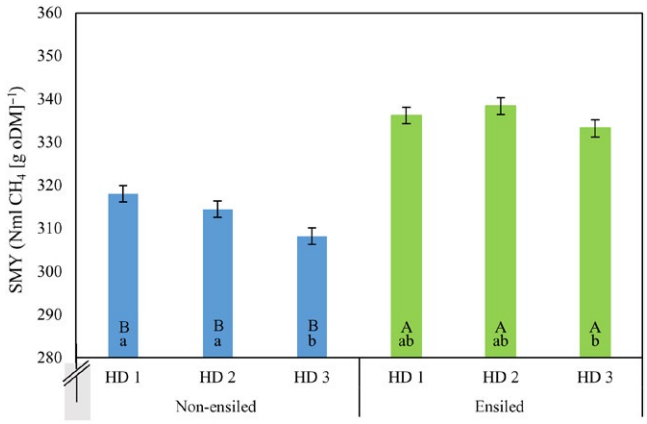

FIGURE 1 Mean substrate-specific methane yield (SMY) [Nml CH_4 (g oDM)$^{-1}$] for harvest date × ensiling. Significant differences between harvest dates (HD 1: mid-September; HD 2: beginning of October; HD 3: mid-October) for non-ensiled biomass (blue bars) and ensiled biomass (green bars) are shown by different lower-case letters. Different upper-case letters indicate significant differences between non-ensiled and ensiled biomass for the same harvest date. Means with identical letters were not significantly different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard errors for SMY

Figure 2 shows the mean values of genotype × harvest date for SMY. Taken as an average of ensiled/non-ensiled biomass, *Sin55* had the highest (337 Nml CH_4 (g oDM)$^{-1}$) and *M×g* the lowest (307 Nml CH_4 (g oDM)$^{-1}$) average SMY of all genotypes. The SMY of *GNT1* remained stable over all three harvest dates, whereas *GNT3* and *Sin55* had a significantly lower SMY at HD 3 than HD 1. *M×g* had its significantly lowest SMY at HD 1.

By way of comparison, the inoculum alone produced 42.87 Nml biogas with a methane content of 43.78%; the maize standard had an average SMY of 356 Nml CH_4 (g oDM)$^{-1}$) (results not shown).

As can be seen in Table 5, the methane hectare yield (MY) was significantly influenced by genotype × harvest date, but not by the ensiling process. Figure 3 presents the mean methane hectare yield for genotype × harvest date. The highest MY (average of ensiled/non-ensiled biomass) was achieved by *M×g* at HD 2 (5,978 Nm^3 CH_4/ha). The lowest average MY was observed in *Sin55* (2,684 Nm^3 CH_4/ha) at HD 1. *GNT3* and *Sin55* had a significantly higher MY at HD 3 than at HD 1. *M×g* and *GNT1* had its highest MY at HD 2 (Figure 3).

3.4 | Velocity of biogas production

The velocity of fermentation of all genotypes, non-ensiled and ensiled, from the three harvest dates is shown in Figure 4. All four genotypes had a considerably lower velocity of biogas production than maize, especially in the first five days of fermentation. On average, *M×g* biomass produced less biogas per hour than the other three genotypes up to day 11. From this day onwards, a similar or slightly higher velocity was observed for *M×g* than the other three genotypes.

The digestion velocity was higher in the ensiled than the non-ensiled biomass of all four miscanthus genotypes in the first few days. Non-ensiled biomass of all genotypes had the highest velocity at HD 1, except *M×g*, which had highest velocity at HD 3. By contrast, for the ensiled

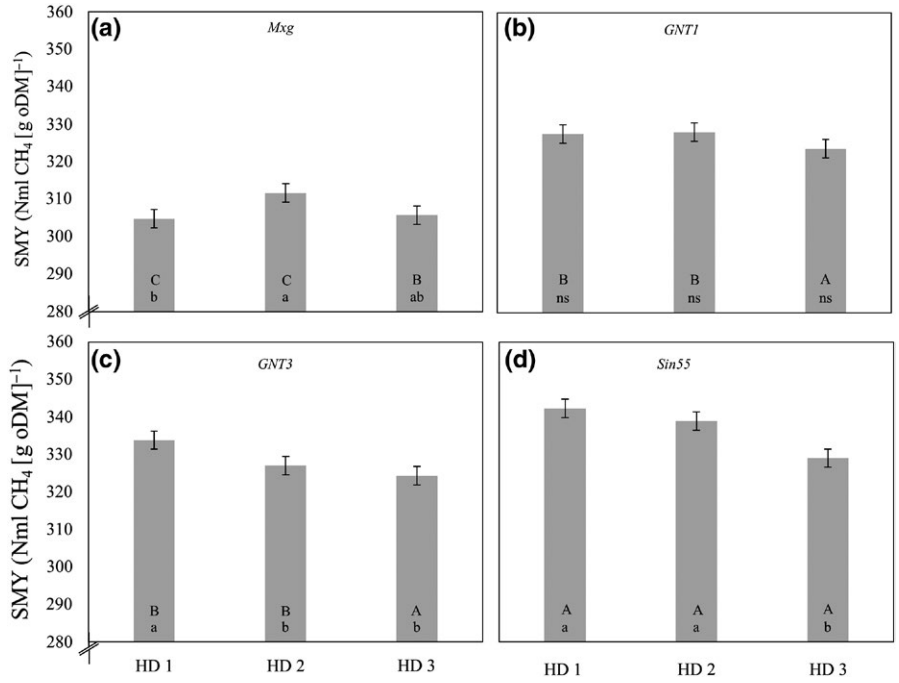

FIGURE 2 Mean substrate-specific methane yield (SMY) [Nml CH4 (goDM)−1] for genotype × harvest date. Significant differences between harvest dates (HD 1: mid-September; HD 2: beginning of October; HD 3: mid-October) for each genotype *(Mxg [a], GNT1 [b], GNT3 [c], Sin55 [d])* are shown by different lower-case letters (ns: not significant). Different upper-case letters indicate significant differences between genotypes for the same harvest date. Means with identical letters were not significantly different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard errors for SMY

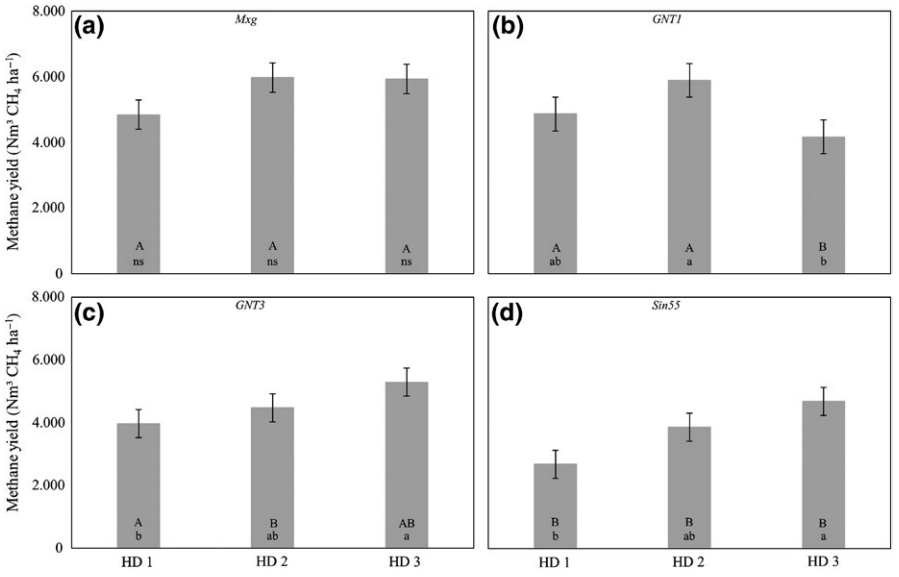

FIGURE 3 Mean methane hectare yield (MY) [Nm3 CH4/ha] for genotype × harvest date. Significant differences between harvest dates (HD 1: mid-September; HD 2: beginning of October; HD 3: mid-October) for each genotype *(Mxg [a], GNT1 [b], GNT3 [c], Sin55 [d])* are shown by different lower-case letters (ns: not significant). Different uppercase letters indicate significant differences between genotypes for the same harvest date. Means with identical letters were not significantly different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard errors for MY.

biomass, a later harvest date was more favourable, as the digestion velocity tended to be higher. It is noticeable that the ensiled biomass of all genotypes from HD 3 behaved similarly to maize, that is, the digestion velocity increased within the first day of fermentation and then decreased again.

4 | DISCUSSION

This study demonstrates that the ensiling of miscanthus biomass is possible without additives and that ensiling positively influences the substrate-specific methane yield and digestion velocity. In addition, it was shown that silage quality varies between genotypes and harvest dates. The following sections discuss the differences in silage quality between the four genotypes and three harvest dates, and also the effect of ensiling on methane yield and digestion velocity. Finally, we give a summary of the results and an outline of what the findings mean for agricultural practice.

4.1 | Ensiling ability of miscanthus biomass

The quality of silage can be measured by various parameters, for example, silage acids and pH value. The two acids, lactic acid and butyric acid, are often used to classify silage quality, as a high level of lactic and low level of butyric acid indicate silage of good quality (Galler, 2011; Liu, Ge, Liu, & Li,

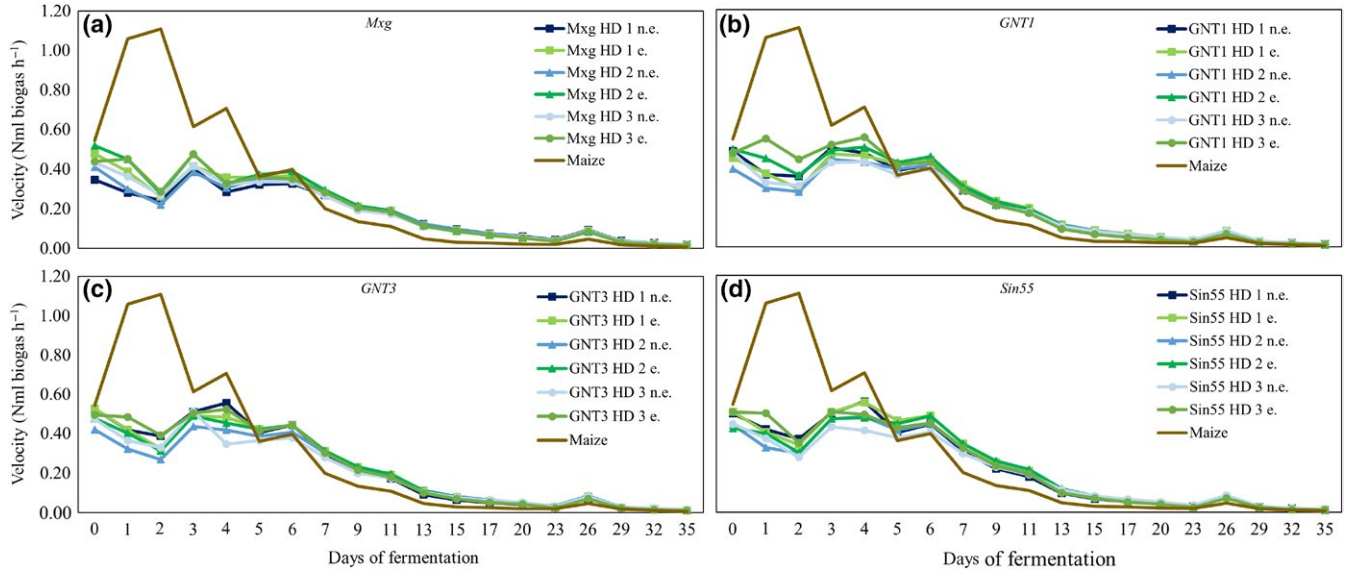

FIGURE 4 Velocity of fermentation [Nml biogas/hr] of the four genotypes (*M×g* [a], *GNT1* [b], *GNT3* [c] and *Sin55* [d]) from the three harvest dates (HD 1, HD 2 and HD 3), both non-ensiled (n.e.) and ensiled (e.) over the fermentation period of 35 days. Non-ensiled biomass is shown in blue colours, ensiled biomass in green colours. HD 1 is indicated by a square, HD 2 by a triangle and HD 3 by a circle. For comparison purposed, the velocity of maize (brown line) is shown as reference

2016). A number of studies recommend a pH value within the range of 3.7–4.5 to achieve a sufficient silage quality (Galler, 2011; Liu et al., 2016; Teixeira Franco, Buffière, & Bayard, 2016; Vervaeren, Hostyn, Ghekiere, & Willems, 2010).

In our study, all genotypes had the highest lactic acid content, lowest butyric acid content and lowest pH at HD 3 (Table 3). Thus, it could be concluded that, in 2017, HD 3 was the best date to harvest miscanthus for ensiling. A comparison between genotypes shows that *M×g* seems to be the most suitable for ensiling, as it not only had highest lactic acid contents, but also lowest butyric contents and the lowest pH (Table 4). By contrast, *Sin55* had the least favourable silage quality values, with the lowest lactic acid contents, highest average butyric acid contents and a comparatively high pH (average 4.91). Therefore, our hypotheses that HD 1 and the stay-green genotype *Sin55* are most suitable for ensiling were not confirmed.

According to Teixeira Franco et al. (2016), higher packing density leads to better silage quality, due to higher lactic acid contents, which makes the silage more stable. With increasing DMC, the compaction of biomass becomes more difficult and thus packing density lower (Baldini et al., 2017). This effect was also shown in our study for the genotype *M×g*; at HD 3, only 550 g biomass could be pressed into the jars, as the DMC had increased compared to HD 1 and HD 2 (600 g fresh biomass). This led to a lower packing density of 366 kg/m^3 at HD 3 compared to 400 kg/m^3 at HD 1 and HD 2. In this study, *M×g* still had the best silage quality at HD 3, despite its lower packing density. However, the higher DMC may lead to compaction problems in agricultural practice.

In our study, the pH value after 3 days of ensiling (indicating speed of pH decrease; results not shown) was significantly lowest for biomass harvested in mid-October (for *GNT1*, it was also lowest but not significantly so). This $pH_{3\ days}$ remained relatively stable until day 90 for each genotype, again showing that mid-October was the most suitable harvest date for miscanthus ensiling in our study.

A possible explanation for the improved silage quality with later harvest dates in our study could be the weather conditions and associated differences in carbohydrate content of the biomass. Purdy et al. (2015) have shown that carbohydrate content in the aboveground biomass of miscanthus fluctuates over the season and can be influenced by weather conditions. In our study, weather conditions at HD 3 did indeed differ considerably from those at HD 1 and HD 2. At and just before HD 3, it was quite sunny and warm, with maximum temperatures around 25°C and minimum temperatures above 8°C, whereas at HD 1 and HD 2, it was cooler with night-time temperatures falling to 4.5 and 5.3°C, respectively. To confirm this hypothesis, further research needs to be performed on the impact of weather conditions on carbohydrate content of aboveground biomass and silage quality.

The differences in silage quality between the genotypes, especially *M×g* and *Sin55*, can probably be attributed to the differences in potassium content. According to Galler (2011), substances with an alkaline effect, such as potassium, lead to poorer acidification. In general, *M×g* had lower potassium and higher lactic acid contents than *Sin55* (Mangold et al., 2019). This might also be a possible

explanation for the improved silage quality with later harvest dates, since miscanthus is relocating minerals such as potassium from the aboveground biomass to the rhizomes with ongoing senescence.

The low butyric acid content of the silage, resulting in a good silage quality, can be explained by the high cutting height of the biomass (in our trial about 20 cm). A higher cutting height leads to a lower ash content and less uptake of bacteria, such as *Clostridium*, in turn leading to lower butyric acid contents (Szymańska, Sulewksa, & Selwet, 2014).

Other studies investigating the ensiling of miscanthus had similar results. Baldini et al. (2017) also harvested miscanthus in mid-October and found similar contents of lactic, acetic and butyric acid to those of our study. The pH value in their study was, however, lower (3.9) than in ours. Whittaker et al. (2016) quantified a lower lactic acid content (about 0.5% of DM), but a higher pH value (5.2) of *M*×*g* harvested in September.

Maize is the most common biogas crop in Germany and known for its good silage quality. Baldini et al. (2017) determined higher contents of silage acids (lactic acid, acetic acid) in maize than in miscanthus. Herrmann, Heiermann, and Idler (2011), however, found similar lactic and butyric acid contents in maize stored for 90 days to those found in our study for *M*×*g* at HD 3. The lactic and acetic acid contents of maize found by Whittaker et al. (2016) were similar to those of all miscanthus genotypes at HD 3 in our study. Therefore, we conclude in our study that, in 2017, HD 3 was the optimal date to harvest miscanthus to achieve similar silage quality results to those of maize.

4.2 | Effect of ensiling on methane yield and velocity

Our study found significantly higher substrate-specific methane yields (SMY) of ensiled than non-ensiled miscanthus biomass for all four genotypes on all harvest dates (Figure 1). This is in line with the results of Amon et al. (2007) and Herrmann et al. (2011), who also demonstrated a positive effect of ensiling on substrate-specific methane yield. Herrmann et al. (2011) found a positive correlation between ensiling products, such as acetic acid, butyric acid and ethanol, and methane content of various crops, which explains the higher SMY of ensiled than non-ensiled biomass.

When calculating the methane hectare yield (MY), Herrmann et al. (2011) emphasize the importance of considering mass losses during the ensiling process. In our study, we found mass losses of up to 7.43% (Supporting Information Table S2), reducing the dry matter yields (DMY) on a per hectare base. Wahid et al. (2015) demonstrated that dry matter yield correlates positively with methane hectare yield. Therefore, the high mass losses of ensiled biomass in our study, which were significantly higher at HD 1 and HD 2 than HD 3 in all genotypes, reduced the MY from these harvest dates. However, these mass losses were compensated for by a higher SMY, ultimately resulting in similar MY for non-ensiled and ensiled miscanthus biomass.

The average SMY (over all genotypes, HD, ensiling) in our trial was 325 Nml CH_4 (g oDM)$^{-1}$, which is higher than data reported in the literature. For example, Baldini et al. (2017) and Mayer et al. (2014) found a SMY for miscanthus ranging between 160 Nml and 250 Nml CH_4 (g oDM)$^{-1}$. Other studies have reported a SMY of up to 309 Nml CH_4 (g oDM)$^{-1}$ and that SMY generally decreases with later harvest dates (Kiesel & Lewandowski, 2017; Kiesel, Nunn, et al., 2017a).

As maize is the most common biogas crop, it is a good benchmark for alternative biogas crops such as miscanthus. The SMY reported for maize ranges between 285 and 400 Nml CH_4 (g oDM)$^{-1}$ (Baldini et al., 2017; Mast et al., 2014; Mayer et al., 2014), which is higher than that measured for miscanthus in our study. The average miscanthus SMY in our study is also lower than that of the internal laboratory maize standard (356 Nml CH_4 (g oDM)$^{-1}$), which is analysed to monitor the activity of the inoculum in each biogas batch test.

The MYs of miscanthus in our study are in the range of the literature values reported for both maize and miscanthus (Baldini et al., 2017; Kiesel & Lewandowski, 2017; Mayer et al., 2014) with the lowest MY for *Sin55* (average: 3,700 Nm^3 CH_4 ha^{-1} a^{-1}) and the highest MY for *M*×*g* (average: 5,500 Nm^3 CH_4 ha^{-1} a^{-1}). This reflects the differences in DMY, which is expected to vary with crop stand age, between these genotypes (see Mangold et al., 2019). Mast et al. (2014) found a MY of 6,000 Nm^3 CH_4 ha^{-1} a^{-1} for maize and Kiesel and Lewandowski (2017) even 6,000 Nm^3 CH_4 ha^{-1} a^{-1} for miscanthus (both studies were conducted in similar environmental conditions to our study).

In addition to high methane hectare yields, velocity of digestion is an important parameter in determining the suitability of novel biogas crops. The faster biomass is digested in a biogas plant, the more efficient the process is. Fast digestible biomass requires less electricity in the fermenter, for example, for stirring, until the substrate has been digested. Moreover, fast digestible substrates theoretically require less fermentation volume, which means the digester size could be reduced to save construction costs (Ward, Hobbs, Holliman, & Jones, 2008).

In our study, the ensiling process influenced the digestion velocity of the miscanthus biomass from all harvest dates. For all genotypes, more biogas was produced in the first nine days of fermentation from the ensiled than non-ensiled biomass (Figure 4). In addition, it was found that ensiled miscanthus biomass tended to have better digestion velocity with later harvest date. However, it was still considerably lower than for maize. In this context, Klimiuk, Pokój, Budzyński, and Dubis (2010) attributed this to the higher lignin content of miscanthus than maize. Fernandes et al. (2009) determined

that a higher lignin content decreases the biodegradability of biomass. The higher lignin content of miscanthus renders the breakdown of cellulose and hemicellulose less efficient than in maize and thus lowers the methane productivity (Klimiuk et al., 2010). However, as Zheng, Zhao, Xu, and Li (2014) pointed out, ensiling can have a positive effect on methane yield and can be seen as a pretreatment for miscanthus biomass. Also Liu et al. (2016) found a higher digestibility for ensiled compared to non-ensiled biomass (giant reed). This explains the higher velocity and specific methane yields of ensiled miscanthus compared to non-ensiled miscanthus. Our study confirmed the hypothesis that ensilaging can serve as a pretreatment for miscanthus biomass with the aim of achieving both faster digestion and a higher specific methane yield.

4.3 | Outlook for agricultural practice

The following summary of the findings of this study considers their practical implications for the utilization of miscanthus biomass in biogas plants.

Firstly, we found out that miscanthus biomass ensiles best when harvested in mid-October. Also methane hectare yield was highest at HD 3 in all genotypes, except *GNT1* (which yielded highest at HD 2). This is a further indication for harvest in mid-October. Our expectation that higher DMCs (later harvest date, genotype-specific characteristics) lead to lower silage quality and methane yields, was not confirmed by this study. The best silage quality and methane hectare yields were both found for a later harvest date and the early (in comparison with the other tested genotypes) senescent genotype *M*×*g*. This leads us to the conclusion that the dry matter content is the most important parameter to consider when determining the optimal harvest date for miscanthus.

Various studies have already recommended harvesting miscanthus in October to give the plant enough time to relocate its nutrients for resprouting in the following year (Kiesel & Lewandowski, 2017; Mangold et al., 2019; Wahid et al., 2015). Thus, in addition to qualitatively better silage, a harvest in mid-October also helps to ensure that enough time is available for relocation of a large fraction of nutrients, which facilitates re-sprouting the following year.

In our study, the miscanthus biomass was milled after ensiling. This generally has a positive effect on the digestibility. However, as the non-ensiled miscanthus biomass was also milled at the same setting in the cutting mill, the observed positive effect can be attributed to the ensiling. Other studies have also found positive effects of ensiling on the methane yield of other crops (Amon et al., 2007; Herrmann et al., 2011). Zheng et al. (2014) suggested ensiling as a pretreatment for miscanthus and various other studies have recommended the pretreatment of miscanthus in general for anaerobic digestion to achieve higher methane yields (Frydendal-Nielsen et al., 2016; Zheng et al., 2014). Such pretreatment, however, is often energy-intensive and therefore associated with high costs (Zheng et al., 2014). In our study, the ensiling step resulted in a higher substrate-specific methane yield (up to 7% on average) and digestion velocity. Therefore, ensiling may save on, or at least reduce, the pretreatment step for miscanthus.

In addition to the various positive effects of ensiling on methane yield, we also found that the ensiling process led to mass losses of up to 7.6% of fresh matter (*Sin55*). However, these mass losses were compensated for by the higher SMY of ensiled miscanthus, resulting in similar methane hectare yields. Whittaker et al. (2016) demonstrated that silage additives reduce mass losses. Therefore, if additives are added to the biomass and mass losses reduced, the positive effect of ensiling may result in higher methane hectare yields. However, ultimately the increase in methane yield should outweigh the additional costs incurred for additives.

In conclusion, we were able to demonstrate that ensiling is suitable to preserve green-harvested miscanthus and even increases its substrate-specific methane yield and digestion velocity. A harvest in mid-October not only improves silage quality, resulting in high hectare methane yields, but also provides sufficient time for relocation of nutrients for regrowth the following year. It may be possible to reduce dry matter losses and further improve the methane hectare yield through the use of additives. These results can help promote the practical implementation of miscanthus as a biogas crop and thus contribute to making biogas production more environmentally benign.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Table S1: Average air temperature and precipitation of the field trial on a monthly basis in the year 2017. Average air temperature was measured 2 m above soil surface.

	Average air temperature [°C]	**Precipitation [mm]**
January	-2.95	38.6
February	4.70	73.3
March	8.48	63.7
April	7.89	68.2
May	14.82	70.4
June	19.66	82.3
July	19.41	147.8
August	20.11	122.0
September	14.24	50.9
October	12.50	75.5
November	6.04	93.8
December	2.41	65.3
Sum	-	951.8

Table S2: Means of silage quality parameters with standard error.

Quality parameter	*Mxg*			*GNT1*			*GNT3*			*Sin55*		
	HD 1	HD 2	HD 3	HD 1	HD 2	HD 3	HD 1	HD 2	HD 3	HD 1	HD 2	HD 3
DMC_{silage} [%]	35.10±0.43	35.25±0.43	39.74±0.43	29.34±0.51	28.14±0.51	33.22±0.51	27.60±0.43	27.74±0.43	32.49±0.43	26.17±0.43	26.51±0.43	33.27±0.43
Lactic acid [% of DM]	1.36±0.29	3.0±0.29	4.53±0.29	0.09±0.34	0.76±0.34	2.87±0.34	0.89±0.29	0.54±0.29	2.20±0.29	0.29±0.29	0.04±0.29	2.63±0.29
Acetic acid [% of DM]	0.43±0.14	0.52±0.14	0.68±0.14	0.72±0.16	0.51±0.16	0.57±0.16	0.97±0.14	1.22±0.14	0.51±0.14	1.08±0.14	1.29±0.14	0.39±0.14
Butyric acid [% of DM]	0.14±0.02	0.09±0.02	0.00±0.02	0.17±0.02	0.16±0.02	0.07±0.02	0.15±0.02	0.16±0.02	0.09±0.02	0.19±0.02	0.20±0.02	0.06±0.02
Ethanol [% of DM]	0.11±0.004	0.12±0.004	0.13±0.004	0.09±0.005	0.07±0.005	0.08±0.005	0.08±0.004	0.08±0.004	0.08±0.004	0.09±0.004	0.08±0.004	0.09±0.004
pH	5±0.08	4.44±0.08	4.08±0.08	5.3±0.09	5.1±0.09	4.4±0.09	5.08±0.08	5.1±0.08	4.61±0.08	5.12±0.08	5.18±0.08	4.43±0.08
Fructose [% of DM]	0.45±0.042	0.47±0.042	0.45±0.042	0.64±0.049	0.69±0.049	0.63±0.049	0.80±0.042	0.72±0.042	0.65±0.042	0.95±0.042	0.94±0.042	0.64±0.042
Buffer capacity*	3±0.24	4±0.24	3.75±0.24	3.67±0.28	4.3±0.28	6±0.28	4.25±0.24	4.5±0.24	4.5±0.24	3.75±0.24	4±0.24	3.75±0.24
Mass losses [% of FM]	5.42±0.40	4.47±0.40	3.0±0.40	7.43±0.48	6.79±0.48	4.46±0.48	6.42±0.40	7.32±0.40	5.12±0.40	7.07±0.40	7.65±0.40	4.55±0.40

5 General Discussion

In this Chapter, the results presented in the chapters 2 to 4 are discussed. Chapter 5.1 focuses on how the results presented in the previous chapters have contributed to make biomass production of miscanthus more efficient and how it can be further improved. In Chapter 5.2 the results of the former chapters are reflected under the question, how miscanthus can be integrated into agricultural production systems most efficiently.

5.1 Efficiency of miscanthus cultivation

In the context of sustainable bioenergy crop production, the environmental benign production and potential positive ecological impacts are important targets, which have to be fulfilled (Lewandowski & Schmidt, 2006). To meet these targets, an ideal bioeconomy crop should have a high and stable biomass yield and a high resource-use efficiency, which includes nutrient-use efficiency, among other characteristics (Pretty et al., 2011; Cosentino et al., 2018).

Especially, nutrient-use efficiency is of great importance in producing environmentally benign bioenergy crops, as fertilization could negatively affect the environment, for instance, by groundwater pollution due to nitrate leaching or by nitrous oxide emissions (Kutra & Aksomaitiene, 2003; de Paz & Ramos, 2004; Roncucci et al., 2015; Monti et al., 2019). Additionally, the production of mineral nitrogen fertilizer requires a high amount of (fossil) energy, while fossil phosphorus resources are limited (Roncucci et al., 2015; Ehmann et al., 2017; Monti et al., 2019).

As fertilization can lead to the mentioned negative environmental impacts, both, environmental efficiency and nutrient-use efficiency are discussed together in Chapter 5.1. Thereby, in the context of this current study, the definition of nutrient-use efficiency consists of three elements: Firstly, it is defined as ratio of biomass yield to nutrient supply, referring to Lewandowski & Schmidt (2006) and Cadoux et al. (2012). Additionally, a high nutrient-use efficiency means in this study, to reduce nutrient losses e.g. via emissions, which is directly linked to the environmental efficiency. The third element defining nutrient-use efficiency is nutrient recycling, for example via leaf fall or digestate application.

Also the definition of environmental efficiency is made up of several elements: as mentioned, a low risk for groundwater pollution, which is caused for example by nutrient leaching, is linked to a high environmental efficiency. In addition to that, an increase in soil quality or a decrease

in greenhouse gas emissions improves it. Furthermore, a high environmental efficiency is linked to ecosystem services such as soil carbon storage.

Following Pretty et al. (2011) *"more output from the same area"* should be produced, as (agricultural) land is a finite resource (Dauber et al., 2012; Fritsche et al., 2015). In the context of the current study, *output* is defined as *yield*. The study of Meyer et al. (2017) has already shown that biomass yield is the most important determinant to reduce greenhouse gas emissions of miscanthus cultivation. Therefore, in this current study, land-use efficiency is defined as yield produced per hectare, which includes dry matter yield as well as methane hectare yield.

Nutrient-use and environmental efficiency

Along the supply chain of miscanthus, there are several possibilities to improve the nutrient-use and environmental efficiency.

Beginning with the establishment, Mangold et al. (2018) have shown that collars are harvested in a non-destructive way from the mother field, which increases the environmental efficiency. This is due to the fact that the most common propagation method (rhizome propagation) goes along with soil disturbance, which could lead to soil erosion or CO_2 losses and thus to a decrease in soil quality (Pimentel et al., 1995; Boersma & Heaton, 2012; Lal, 2015). As collar harvest can be conducted without soil disturbance, this mitigates the risk for soil erosion and CO_2-losses and thus increases environmental efficiency.

Additionally, miscanthus is not able to use all available nutrients in the establishment year, which may lead to losses via emissions or leaching (Cadoux et al., 2012; McCalmont et al., 2017). These losses are likely the result of the low planting density of miscanthus, which ranges between 1-4 plants per m² (Lewandowski et al., 2000). To reduce nutrient losses and thus improve the nutrient-use and environmental efficiency, miscanthus could be established under a second crop. This could reduce nitrate leaching, as shown in the study of Whitmore & Schröder (2007), who have undersown grass in maize. The establishment of a perennial under an annual crop is also proceeded in other perennials, for example in cup plant (Heimler & Fritz, 2017). As this approach could also improve the land-use efficiency, this will be discussed below.

In the study of Mangold et al. (2019a) it is shown that the average nutrient removal of green harvested miscanthus is 115 kg ha^{-1} a^{-1} for nitrogen, 257 kg ha^{-1} a^{-1} for potassium and 17 kg ha^{-1} a^{-1} for phosphorus. It was shown that delaying the harvest date of miscanthus from

mid-September to mid-October increases the nutrient-use efficiency (Mangold et al., 2019a). To close the nutrient cycle and thus increase the nutrient-use efficiency, nutrients could be brought back to the field via digestate application, if miscanthus is used as biogas substrate. By doing so, the nutrient removal trough harvested biomass could be at least partly restored in an organic way, which lowers the demand for mineral fertilizer and thus positively affects the availability (phosphorus) or reduces the production capacity (nitrogen).

To increase the environmental efficiency, biogas digestates could be separated and then applied to the field, as suggested by Ehmann et al. (2018). Unseparated digestates have a high water content and thus a high volume (Ehmann et al., 2018), which results in high costs for storing and transportation. The solid fraction includes approx. 20% of total nitrogen, 30% of total phosphorus and 15% of potassium (Ehmann et al., 2018), making it a valuable fertilizer. Therefore, miscanthus could be fertilized with this solid fraction. By doing so, the nutrient cycle is closed and environmental efficiency of digestate fertilization could be increased, as less water has to be transported and thus less greenhouse gases are emitted. However, as stated by Ehmann et al. (2018) the solid fraction should be incorporated after its application to avoid nitrogen-emissions. This is one of the main challenges as it is not possible in miscanthus due to its stubbles. Therefore, similar to the suggestion of Ehmann et al. (2018) for grassland, on miscanthus stubbles (presently) only the liquid fraction of digestates can be recommended for an application, as solids are limited by application practicability.

For brown harvested miscanthus, Roncucci et al. (2015) and Ruf et al. (2017) found a lower nutrient demand compared to green harvest, which is in the range of 72 - 80% less nitrogen, 48 - 65% less phosphorus and 52 - 65% less potassium. This lower demand is due to a lower removal of nutrients at harvest via biomass because of leaf fall and nutrient translocation to the rhizome during winter (Cadoux et al., 2012). Cadoux et al. (2012), recommended to fertilize brown harvested miscanthus with 73.5 kg nitrogen ha^{-1}, 7.0 kg phosphorus ha^{-1} and 105.0 kg potassium ha^{-1}, if yield level is about 15 t ha^{-1}. However, they emphasise soil analyses before fertilizers are applied to determine the exact demand for fertilizer and thus avoid losses.

Additionally, Cadoux et al. (2012) recommend to fertilize miscanthus only in its growing phase when it is able to take nutrients up, which is about one month after shoot emergence at the earliest, to avoid inefficiencies and losses (Cadoux et al., 2012).

Although many studies were conducted to analyse to what extent fertilization improves miscanthus yield, this topic is not finally clarified, as the exact fertilizer requirements during growth cycle have not been identified yet (Cardoux et al., 2012). The ideal fertilization level

will meet the demand of the crop and also avoids nutrient losses (Cardoux et al., 2012). In the review of Monti et al. (2019) it is illustrated that fertilization rates for miscanthus range from 0 - 220 kg N ha^{-1}, while yield increase ranges from -40% to +120%, which underlines the importance of correct fertilization: if too little fertilizer is applied, a yield decrease might be the consequence. On the other hand, overfertilization could lead to nutrient losses. As fertilization effects are likely to depend on environmental and soil conditions, as well as crop management and water availability (Monti et al., 2019), further research is necessary to investigate the effect of fertilizer on yield increase to give more precise fertilizer recommendations, as it was already stated by Cadoux et al. (2012).

At the removal of miscanthus, also a high environmental and nutrient-use efficiency is desirable. In the study of Mangold et al. (2019c), miscanthus was harvested in February. Afterwards, the field was ploughed to a depth of 20 cm and then harrowed. Finally, crops were sown and commonly cultivated. If miscanthus is removed after the harvest in February, soil is covered over winter, which reduces soil erosion and nutrient depletion and thus increases environmental efficiency. Additionally, nutrients of miscanthus biomass have been recycled via leaf-fall over winter and are thus provided through the soil for subsequent crops, which is why less fertilizer has to be applied and nutrient-use efficiency could be increased. To fertilize subsequent crops with less fertilizer was also recommended in the study of Mangold et al. (2019c): it was shown that soil N_{min} content increased after a miscanthus removal, as some nitrogen was supplied by the decomposition of miscanthus residues. However, it was generally on a low level as nitrogen was partly fixed in organic matter of miscanthus residues (Mangold et al., 2019c). If less fertilizer has to be applied for the subsequent crop, (mineral) fertilizers can be saved and overfertilization reduced, which enhances nutrient-use and environmental efficiency. However, as the removed miscanthus of Mangold et al. (2019c) was not fertilized, further studies should clarify, if the given recommendation for subsequent crops is also true, if the removed miscanthus had been fertilized during its productive phase, as then possibly more nitrogen available for plants is provided by the soil.

Referring to the study of McCalmont (2018), a miscanthus removal has to be conducted under consideration of soil nitrous oxide fluxes. In their study, miscanthus had a N_2O flux of about 0.13 mg m^{-2} h^{-1} in the first year of miscanthus removal, which started with a glyphosate spraying. In the following period, which began after sowing of perennial ryegrass, N_2O flux decreased to 0.08 mg m^{-2} h^{-1}. In the period, in which N_2O flux was measured, several peaks of N_2O fluxes were observed (McCalmont et al., 2018). Thereby, it was remarkable that the three highest peaks occurred at glyphosate application, at soil cultivation and sowing of forage kale,

and at the fertilization of perennial ryegrass. Those N_2O fluxes have led to an increase of the carbon costs by 3.41 t CO_2 eq. ha^{-1} for the lifetime of the miscanthus grown in the study of McCalmont et al. (2018). This, however, reduces the environmental and nitrogen-use efficiency of miscanthus. Therefore, some management practices, as suggested by Baggs et al. (2000) could be applied to reduce N_2O emissions of miscanthus removal and thus improve the nitrogen-use and environmental efficiency. In the study of Baggs et al. (2000), the temporarily increased N_2O emissions after soil tillage were attributed to the decomposition of plant residues and an increased gas diffusivity due to soil disturbance. They recommended to *'synchronize nutrient release and crop demand'*, which means that crop residues incorporation and soil tillage should take place just before the following crop is sown (Baggs et al., 2000). Additionally, they suggest avoiding fallow land and rather propose to grow catch crops, which can take nitrogen of decomposed plant residues up (Baggs et al., 2000). The subsequent crop following miscanthus (either main crop or catch crop) could take the available nitrogen up, which means in turn that less nitrogen can get lost via nitrification/denitrification and thus via N_2O emissions. By doing so, nutrient-use and environmental efficiency can be improved.

Land-use efficiency

A high land-use efficiency is crucial for the decision, which crop should be grown, especially, in areas with a high pressure on agricultural land. Pressure on (agricultural) land increased as, for example, biogas plants were built in regions with a high livestock density in Germany (BMEL, 2015). Thus, different end-uses of biomass (in this case for bioenergy or fodder) compete against each other for the same agricultural land. Therefore, Pretty et al. (2011) postulated to produce *"more output from the same area"*.

Miscanthus, however, is usually not harvested in the first year. The grown biomass of the establishment year is often mulched, because of its low biomass amount (Witzel & Finger, 2016). This 'yield loss' reduces not only the total land-use efficiency of miscanthus, it might also hinder farmers to cultivate miscanthus, as no yield is generated in the first year.

One possibility to overcome this 'yield loss' could be to establish miscanthus under an annual crop such as maize, as already mentioned in the sub-chapter '*nutrient-use and environmental efficiency*'. The idea of this approach is, to establish miscanthus under a crop producing a high biomass amount, which is harvested in the first year. From its second growing season onwards, miscanthus has built enough biomass, which can then be harvested annually. Consequently, a

continuous yield could be generated from the establishing year onwards and thus a higher output was generated from the same area.

Cossel et al. (2019) have shown that establishing miscanthus under maize is possible and that the accumulated biomass yields (maize + miscanthus) over three years of this establishing method (1st year: maize + miscanthus, 2nd and 3rd year: solely miscanthus) could be nearly twice as high compared to a solely grown miscanthus stand (1st to 3rd year miscanthus). This considerable difference can be attributed to high maize yields in the establishment year, which represent one third of total accumulated yields (Cossel et al., 2019). Miscanthus yields in the maize + miscanthus plots were significantly lower in the establishment year (2016) and the following year (2017) compared to the miscanthus reference (solely grown miscanthus) but were not significantly different anymore in the third growing season (2018) (Cossel et al., 2019). By investigating some cultivation aspects, for example maize density, the establishment of miscanthus under maize could help to improve the total land-use efficiency of miscanthus and make it more attractive for farmers.

But not only at the establishment, already by producing miscanthus propagation material a higher land-use efficiency could be achieved, as shown in the results of Chapter 2: It was shown that miscanthus propagation via collars has a higher land-use efficiency than rhizome propagation. This is due to the non-destructive harvest of collars by pulling out the stems (Mangold et al., 2018). By doing so, rhizomes remain in the field, which allows a resprouting of miscanthus and thus ensures a yield of the mother field. In contrast, in rhizome propagation the mother field is ploughed and rhizomes are (partly) removed, which set the field back to 'year one' (year of establishment; Boersma & Heaton, 2012) and thus lower the yield.

Furthermore, the genotype selection can increase land-use efficiency, as shown in the studies of Chapters 4.1 and 4.2. It was illustrated that methane hectare yield (MY) was mainly affected by dry matter yield, which is why *Mxg* had the highest MY (Mangold et al., 2019a). Additionally, in Mangold et al. (2019b) it was shown that the biomass of *Mxg* had the best silage quality with the lowest mass losses. However, it was indicated that leaf-rich genotypes, such as *Sin55*, had a significantly higher substrate-specific methane yield (SMY) than the standard cultivar *Mxg* (Mangold et al., 2019a). Hence, miscanthus breeding for biogas production should focus on the genotype characteristics *high yield* and *leaf-richness*, which in combination lead to high MY and thus increase the land-use efficiency of miscanthus.

If miscanthus is successfully established, the choice of harvest date can also improve land-use efficiency, as the results presented in Chapters 4.1 and 4.2 have illustrated. It was shown that

the optimal harvest date for a green cut was mid-October (Mangold et al., 2019a,b). This harvest date ensures a sufficient nutrient recycling to the rhizomes, which secures the long-term yields and thus a high land-use efficiency of miscanthus. In addition to that, this harvest date has led to a good silage quality with the lowest mass losses and the highest methane hectare yields, which enhances land-use efficiency (Mangold et al., 2019b).

Also at the removal of miscanthus, the land-use efficiency can be increased, as shown in the study of Mangold et al. (2019c). Maize not only supressed satisfactorily resprouting miscanthus, it also yielded highest and was therefore recommended to be grown after miscanthus (Mangold et al., 2019c). If other crops than maize are cultivated after a miscanthus removal, these should suppress resprouting miscanthus efficiently to generate high yields and thus to ensure a high land-use efficiency.

A further possibility to remove miscanthus and simultaneously increase land-use efficiency is described in the following approach: as recommended by Mangold et al. (2019a,b), biomass could be harvested in October and used for example as biogas substrate. Afterwards the miscanthus stand could be removed via ploughing to bring the rhizomes to the top soil layer. Over winter, when temperatures are decreasing, rhizomes might be damaged, which lead to lower resprouting rates in the following year. Referring to this, Clifton-Brown & Lewandowski (2000) showed in their study, that the lethal temperature for *M. x giganteus* and *M. sacchariflorus* genotypes is -3.4°C, while it is for *M. sinensis genotypes* -6.5°C. This approach to remove miscanthus increases also efficiency, as the natural effect of freezing is used and thus with a comparatively low input (ploughing) a high damage of rhizomes can be achieved. Therefore, future research should investigate, if miscanthus could be removed this way. However, it has to be mentioned that this approach presumes a possibility for the farmer to utilize the green miscanthus biomass.

Another approach, to remove miscanthus efficiently and to ensure a 'clear' field for the subsequent crops is shown in the studies of Dufossé et al. (2014) / Drewer et al. (2016)[1] and McCalmont et al. (2018). In those studies, the basic idea of removing miscanthus was the same: it was tried to damage the regrown miscanthus in its most sensitive phase (in each study June), when the plant builds a high amount of biomass. In this context, a study of Purdy et al. (2015) has shown that starch and soluble sugar content in rhizomes decreased to their lowest amount in May to July, while the content of both was highest in above ground biomass in the same period for almost all genotypes at both tested sides. This in turn means, that if miscanthus is cut

[1] The studies of Dufossé et al. (2014) / Drewer et al. (2016) are based on the same field trial.

in this period, as conducted in the studies of Dufossé et al. (2014) / Drewer et al. (2016) and McCalmont et al. (2018), rhizomes are strongly weakened. Thus, efficiency is increased, as with a low input (in this case glyphosate) miscanthus can be damaged strongly, due to its high sensitivity. By damaging miscanthus that strongly, the subsequent crop is likely less impaired and thus yield is not negatively affected by resprouting miscanthus, which could result in higher yields and a higher land-use efficiency (compared to subsequent crops, which struggle under resprouting miscanthus). However, as the application of glyphosate is feared to affect the environment negatively, this approach could decrease the environmental efficiency.

In the context of producing a high yield and thus improving land-use efficiency, Witzel & Finger (2016) reviewed several studies to analyse the productive lifetime of miscanthus. For continental Europe, its productive lifetime varied between 10 and 21 years, with a median of 16 years (Witzel & Finger, 2016). If then miscanthus has to be removed due it is low productivity, but a new miscanthus field is planned to be grown on the same field, an alternative to removing would be to plough the field after harvest to stimulate plant regrowth. Deng et al. (2013) mentioned in their study that clonal fragments from deep soil layers are able to build a new plant if they were taken up by soil tillage such as ploughing. They demonstrated that 80% of large buds (>0.80cm) of *M. sacchariflorus* from the top soil layer (0-10cm) sprouted, 65 days after they were dug out. From deeper soil layer (10-20cm) around 60% of large buds sprouted (Deng et al., 2013). In smaller buds 65% resprouted from the top layer (0-10cm) while only 25% from the deeper layer (20-20cm) resprouted (Deng et al., 2013).

If farmers stimulate the regrowth of low productive miscanthus by ploughing, a higher yield would probably be possible, compared to a completely new establishment of miscanthus, which in turn would lead to a high land-use efficiency. Additionally, this approach would increase the resource-use efficiency, as with less effort (ploughing) a new miscanthus stand could be established.

5.2 Integration of miscanthus into agricultural production systems

The integration of miscanthus into agricultural production systems should take place on different *levels*: First of all, farmers should have *market options* for the miscanthus biomass. Either, miscanthus can be used on the farm for several utilization pathways or it can be sold on various markets. Secondly, the question arises, on which *land* farmers can grow miscanthus: It could be grown on available (marginal) agricultural land 'within' the farm, which is for example difficult accessible land. Another option could be to grow miscanthus 'outside' of the farm on reclaimed lignite soils. By doing so, these soils could be recultivated to agricultural land. Furthermore, to integrate miscanthus into agricultural production systems, it should fit into the *operation procedures* of other farm activities. Finally, to produce environmentally benign biomass, the cultivation of miscanthus should also fulfil *ecosystem services* to *"increase the flow of environmental services"* (Pretty et al., 2011).
In this Chapter 5.2, the integration of miscanthus into agricultural production systems is considered on the basis of the previously mentioned four levels, that is *i) miscanthus utilization pathways*; *ii) possible land types to cultivate miscanthus on*; *iii) integration of miscanthus into farm operation procedures* and *iv) environmental services of miscanthus*. Thereby, results of former chapters and new aspects are discussed in the different sub-chapters.

Miscanthus utilization pathways

If miscanthus is cultivated as crop on agricultural farms, it should match the structures of a farm, which means that there should either be the possibility for farmers to use the miscanthus biomass themselves or to have access to potential end-markets. Thus, the following sub-chapter outlines possible end-uses of miscanthus and how those can be integrated into the agricultural farm business.

In the studies of Mangold et al. (2019a,b), miscanthus was proven as biogas substrate. It was shown that miscanthus can reach a methane hectare yield of more than 5,200 m^3 CH_4 ha^{-1} a^{-1}, which is at the lower end of the range for maize (Mangold et al., 2019a). Additionally, it was shown that miscanthus can be successfully stored by ensiling (Mangold et al., 2019b). Ensiling of miscanthus has not only led to an increase of substrate-specific methane yield, it also improved digestion velocity (Mangold et al., 2019b). As already stated in Mangold et al. (2019b) a faster digestion increases the resource-use efficiency of biogas substrates: for example, less stirring is necessary, which has to be conducted to stimulate gas formation and to avoid floating layers (Ward et al., 2008). Thus, energy is saved. Additionally, if substrates

"digest" faster, a comparatively lower fermentation volume is necessary, which in turn reduces the demand for building material for the fermenter, and thus resources and energy. In summary, a fast digestible biomass improves as well the energy and the resource-use (energy, building material) efficiency and thus reduces biogas production costs.

Although miscanthus was shown to be a promising biogas substrate, it probably will not be used as solely substrate but in a mixture. In biogas plants often a mixture of several energy crops and/or manure as substrate is used (Dederer & Messner, 2011). A cofermentation of miscanthus together with maize, for example, would have several advantages. As both crops are harvested in the same period in autumn, they could be ensiled together. This was suggested by Gansberger et al. (2015) for *Silphium perfoliatum* L., which is another promising perennial biogas crop. Their suggestion of a joint ensiling of cup plant together with maize could be transferred to miscanthus and lead to an increase of silage quality, as maize is very suitable for ensiling (Gansberger et al., 2015; Baldini et al., 2017).

But also other silage mixtures are possible, as shown by Garmeister et al. (2018): In their study, sugar beet was ensiled together with cereal straw. It was shown that ensiling served as pre-treatment of straw and has led to a better biodegradability, as hemicellulose was hydrolytically broken down into water-soluble carbohydrates (Garmeister et al., 2018). Additionally, by adding straw to sugar beet, the dry matter content in this silage-mixture was increased and thus silage-losses of sugar beet, which can amount up to 40% due to leaking silage effluent, were reduced (Garmeister et al., 2018). This in turn has led to similar methane yields of this mixture compared to maize (Garmeister et al., 2018).

As a lignocellulosic biomass, brown harvested miscanthus has similar characteristics to straw. Thus, brown miscanthus biomass could be ensiled together with sugar beet to improve methane yields of both substrates. By delaying the harvest date of miscanthus after winter, nutrient-use efficiency can be increased. This was shown in the study of Mangold et al. (2019a), who could show that delaying the harvest date by four weeks in autumn already has increased nutrient-use efficiency. In addition to that, a delayed cut of miscanthus can also contribute to enhance the lifetime of its plantation ensuring the long-term yields, as stated by Mangold et al. (2019a). This, in turn, would increase the land-use efficiency of miscanthus. Additionally, energy and costs can be saved, as pretreatment for miscanthus biomass is performed by ensiling, as it was shown in the study of Mangold et al (2019b). Finally, the land-use efficiency of sugar beet could be increased, as losses through ensiling are reduced, which positively affects the methane hectare yield.

It has to be mentioned that sugar beet harvest and brown harvest of miscanthus possibly do not take place at the same time, as sugar beet is normally harvested in November/December and miscanthus in the period from December to March in Germany (Kohl & Heimbach, 2018; Lewandowski et al., 2000). As sugar beets, however, are often stored in a clamp at the field, they could be stored until miscanthus harvest takes place. In the study of Kohl & Heimbach (2018), it was shown that a storage of sugar beet (harvested on 1st December) for 105 days until March has led to losses in sugar yield of about 11.2%. If sugar beet would be stored until the miscanthus harvest in February, the sugar losses could be reduced and would then amount 5.6% (Kohl & Heimbach, 2018). Despite the fact that this approach requires sugar beets to be stored at the field in a clamp, the losses in sugar (approximately 11.2%) and of total sugar beets (8.1%) even at miscanthus harvest in March are lower compared to a single ensiling of sugar beets, which leads to losses of up to 40% (Garmeister et al., 2018).

One idea of bioeconomy is to utilize biomass in *cascades* (BMBF & BMEL, 2015). The idea of this approach is to use biomass several times, beginning to use it for a higher value application (material-use) and re-use it then in an energetic way (Besi & McCormick, 2015). As the same biomass is then used for a material and an energetic purpose, this approach increases resource-use efficiency of biomass (Besi & McCormick, 2015). In this context, the study of Garmeister et al. (2018) has also shown an interesting approach: They have ensiled sugar beet together with horse manure, which resulted in an average biogas yield of 569 m^3 $(t\ oDM)^{-1}$, which was 73% of the biogas yield of the control silage maize (Garmeister et al., 2018). Therefore, another approach to increase the efficiency of miscanthus could be to use it first as animal bedding, ensile the manure afterwards with sugar beet and then use the mixture as biogas substrate in a biogas plant with combined heat and power. By doing so, not only the resource-use efficiency of miscanthus can be increased, as it is used twice: as animal bedding and later as biogas substrate. Additionally, for animal bedding miscanthus has to be harvested brown after winter, which increases both, nutrient-use efficiency and land-use efficiency, as stated above.

In the context of animal bedding, different studies have shown the advantages of miscanthus as litter compared to straw or wood chips for sheep, horses, cows and turkeys (Van Weyenberg et al., 2016; Rauscher and Lewandowski, 2016; Muskowitz, 2017; Lewandowski et al., 2018). Main advantages are a better capacity of water absorptiveness and ammonia adsorption of the miscanthus litter, which goes ahead with a lower demand, as it has to be renewed less often (Rauscher and Lewandowski, 2016; Muskowitz, 2017; Lewandowski et al., 2018). Additionally, referring to Lewandowski et al. (2018) poultry such as turkeys were healthier if

they were kept on miscanthus litter. This, in turn, could save costs for medicine or veterinary. Van Weyenberg et al. (2016) has shown that miscanthus raises less dust than straw litter, which additionally preserves health of animals and farmers. Furthermore, Rauscher & Lewandowski (2016) stated that miscanthus litter requires less manure storage, due to its lower mass and volume compared to straw or woodchips.

In summary, this approach of using miscanthus in cascades can considerably increase the land-use efficiency of miscanthus, as it is used as bedding material, biogas substrate and additionally improves methane yield of sugar beet. By fertilizing fields with the digestate of the biogas plant, additionally, the nutrient cycle can be closed and thus nutrient-use efficiency increased. The mentioned positive aspects of miscanthus as animal bedding can even improve the health of farmers and animals. In addition to that, if farmers use miscanthus, which is grown for animal bedding, straw residues of the remaining agriculture land could remain on the field. This, in turn, likely increases soil organic carbon of these fields (Mahmoodabadi & Heydarpour, 2014). Miscanthus cultivation itself leads to an increase in soil organic carbon but still provides biomass (McCalmont et al., 2017). Thus, an extra growing of miscanthus for animal bedding could contribute to an increase of soil organic carbon on agricultural land and thus improve soil fertility on the total land of a farm. However, further research should investigate, if the results of Garmeister et al. (2018) (ensiling sugar beet together with horse manure from cereal straw) could be transferred to animal bedding based on miscanthus straw and if this is also applicable for other animals than horses.

A further approach of using miscanthus in cascades illustrates the research project ‘BioC4’: In a first step, miscanthus is used to produce the platform chemical bio-isobutanol (FACCE-SURPLUS, 2019). The residues appearing during fermentation is then digested in a biogas plant (FACCE-SURPLUS, 2019). By using miscanthus this way, biomass would also be used twice and thus the total efficiency is increased, as a higher output (isobutanol plus combined heat and power in a biogas plant) with the same input could be generated.

Possible land types to cultivate miscanthus on

If famers decide to grow miscanthus, they have to decide on which agricultural land they will cultivate it. In this context, several studies have illustrated that miscanthus could be grown on marginal lands (Dauber et al., 2012; Xue et al., 2016; Krzyżak et al., 2017; Mehmood et al., 2017; Wagner et al., 2019). As stated above, those marginal lands could be found 'within' a farm or 'outside' of a farm. Referring to Clifton-Brown et al. (2017), growing miscanthus on marginal land could increase the total efficiency of an agricultural farm, as the resources remaining from the lower marginal land effort could be spend to more productive land, likely resulting in increasing yields on these fields (Clifton-Brown et al., 2017). If miscanthus is grown on these marginal lands and satisfying yields could be achieved, with less effort compared to annual crops, the total farm efficiency would increase even further.

As the term 'marginal lands' contains various (soil) characteristics, it is defined as follows: From an economical point of view, land is marginal if yield and realised price are not sufficient to cover the production costs (Dale et al., 2010; Kang et al., 2013). The reason for that could be physical and/or chemical soil problems in general or lands, which are negatively affected by climatic conditions (Dauber et al., 2012; Blanco-Canqui, 2016). The term 'marginal' can also include lands, which are *"idle, under-utilized, barren, inaccessible, degraded, excessed or abandoned"* (Dale et al., 2010). But also highly erodible lands, flood-prone-, contaminated-, acid- and saline- or compaction-prone soils count to marginal lands (Blanco-Canqui, 2016).

According to Blanco-Canqui (2016) the growing of dedicated perennial grasses, such as miscanthus, on marginal lands provides several ecosystem services as *"soil water and wind erosion control, soil carbon sequestration, absorption or retention of pollutants or metals, stabilization or reclamation of mine-soils and improvement of soil properties"*. In this context, the study of Wagner et al. (2019) has shown that from an economic and ecological point it can be lucrative to cultivate miscanthus on degraded lands, as soil could be restored. However, the economic viability is restricted by dry matter yield, which should at least amount 11 t DM ha^{-1} a^{-1} (Wagner et al., 2019).

Clifton-Brown et al. (2017) suggested growing miscanthus on unshaped fields, which badly can be cultivated, especially with an increasing bigger machinery. Such fields could be found 'within' a farm. Growing annual crops on such fields would lead to inefficiencies, as each cultivation procedure (soil cultivation, sowing, fertilization et cetera) is labour time-consuming. If miscanthus was grown on these unshaped fields, mainly in the first year plenty of time has to be spend for its establishment. In the following vegetation periods, however, miscanthus

needs less labour input, which makes it more efficient compared to annual crops on unshaped fields.

In addition to that, Clifton-Brown et al. (2017) proposed miscanthus to be cultivated on fields with a high weed pressure. By growing miscanthus on such fields, weed pressure could possibly decrease as miscanthus suppresses weeds through its compact growth.

As mentioned above, reclaimed lignite mine soils are also possible land to grow miscanthus. Those soils could be found 'outside' of a farm. In Germany, the total mining area is about 85,000 ha and further 30,000 ha are approved for it, referring to Krümmelbein et al. (2012). For recultivation of those areas, there are three opportunities: agricultural, forestal or agroforestry recultivation (Krümmelbein et al., 2012). For agricultural recultivation on lignite mine soils, Krümmelbein et al. (2012) recommend a crop, which cycles nutrients, uses water in an efficient way, develops deep rooting systems and produces high above and below ground biomass. Regarding to those criteria, miscanthus seems to be suitable. In this context, the study of Jeżowski et al. (2017) has shown that miscanthus can be successfully grown on waste heaps of former lignite mines. It was shown that miscanthus (*Mxg*), fertilized over three years with a total amount of 400 t ha^{-1} fresh matter of sewage sludge, reached a yield of 15 t DM ha^{-1} in the third cultivation year (Jeżowski et al., 2017). The high fertilization level, which was four times higher than in common agricultural practice and applied to improve the poor soil characteristics, probably negatively affects the environment (Jeżowski et al., 2017). Therefore, it was noted that the yield of 15 t DM ha^{-1} could possibly be achieved by a lower fertilizer input (Jeżowski et al., 2017). However, those results are very promising: on the one hand, land which could probably not be used for food or feed production due to its contamination with heavy metals (Jeżowski et al., 2017), can be used for biomass production and thus mitigate land-use conflicts between food/feed/fuel production. On the other hand, growing bioenergy crops on such areas could improve landscape and generate new jobs (Krümmelbein et al., 2012). Additionally, miscanthus could be used for energy production in an environmental efficient way via combustion or anaerobic digestion. Furthermore, growing perennial crops on such land can improve soil quality, as it was shown in the study of Matos et al. (2012) for *Robinia pseudoacacia* L.. Thus, growing miscanthus on former lignite mine soils could be a sustainable (which means ecological, economic and social) solution to recultivate those areas.

Integrating miscanthus into the farm operation procedures

As mentioned above, the cultivation of miscanthus also has to fit to the operation procedures of the remaining farm activities. Miscanthus is established after the last spring frosts in April/May (Fritz & Formowitz, 2009), which is coincident to the sowing of maize. Subsequent cultivation measures in the establishment year, for example weeding or plant protection measures, fall into the same period as for other spring crops and maize. However, as the establishment of miscanthus has only to be conducted once in its lifetime, this could contribute to a reduction of work peaks. If miscanthus biomass is harvested green in autumn, a harvest in mid-October was recommended (Mangold et al., 2019 a,b). As this harvest date falls in the range of maize harvest, the ensiling of miscanthus together with maize, as it is stated above, is possible. If miscanthus is harvested brown after winter, this harvest would take place in the period from December to March in Germany (Lewandowski et al., 2000). The period over winter is less suitable to work on the field due to the weather conditions in Germany. Therefore, a brown harvest of miscanthus could help to reduce work peaks even further, in contrast to a green harvest.

Environmental services of miscanthus

As already mentioned in the general introduction of this study, miscanthus provides several ecosystem services, such as carbon storage and CO_2-mitigation potential or its positive effects on species and habitat diversity compared to annual crops (Asbjornsen et al., 2014; McCalmont et al., 2017). Therefore, these environmental services could be integrated into agricultural production to improve the environmental efficiency.

As an example, the study of Dauber & Miyake (2016) has shown an opportunity to a solely cultivation of miscanthus on fields. It was suggested, to grow miscanthus in combination within other (annual) energy crops, for example in strips (Dauber & Miyake, 2016). By doing so, the perennial buffer strips could enhance the biodiversity of arable lands and lead to an increase in soil organic carbon storage (Dauber & Miyake, 2016). Additionally, as perennial crops cover the field over the whole year, soil erosion, nutrient leaching and drift of plant protection to natural habitats can be reduced (Dauber & Miyake, 2016). Hence, the environmental-efficiency of the field can be increased and yield is still generated.

Additionally, miscanthus was recommended and since the year 2018 also included as a so-called *greening measure* within the European Common Agricultural Policy (Emmerling & Pude, 2017; EU, 2017). This greening measure is compulsory for farms with more than 15 ha

agricultural land and obligates to use 5% of the arable land for ecological focus areas (Landwirtschaftskammer NRW, 2019). To fulfill this requirement, several measures are possible, such as abandonment of land for one year, undersowing crops within a main crop or cultivate catch crops between the vegetation of two main crops (Landwirtschaftskammer NRW, 2019). Each of those measures is assessed with different factors to categorize their ecological effectiveness, ranging from 0.3 to 1.5. By abandoning arable land for one year, for example, the ecological value is 1.0 (Landwirtschaftskammer NRW, 2019). However, it is not allowed to use the grown biomass from this land, which in turn reduces land-use efficiency. Undersowing and catch crops have a comparatively lower ecological efficiency and thus a factor of 0.3 (Landwirtschaftskammer NRW, 2019). For both measures, however, it is allowed to harvest the grown biomass once a year, which leads to a better land-use efficiency compared to abandoned land. Miscanthus as greening measure has a factor of 0.7, which is due to its comparatively high ecological efficiency. Additionally, it is allowed to harvest miscanthus biomass for several utilization pathways. Thus, growing miscanthus as a greening measure allows farmers to increase the environmental and land-use efficiency.

In Baden-Württemberg, since 1 January 2019 it is not allowed anymore to use the 5 m area next to a watercourse as arable land (Meier et al., 2018). In this area, it is allowed to grow permanent grassland, perennial wild plant mixtures, arable fodder grasses, cup plant or short rotation coppice (Meier et al., 2018). As shown in the studies of Feldwisch (2011) and Ferrarini et al. (2017), the cultivation of miscanthus on riparian buffer stripes could reduce soil erosion and nutrient run-off and thus serve as water protection. Therefore, it is recommended, to include miscanthus in the list of allowed crops to be grown on riparian buffer stripes. Miscanthus does not have to be fertilized and weed control could be done mechanically. As miscanthus is also a low-input crop and thus similar to cup plant or short rotation coppice, it could provide a higher variety for famers for possible crops, which are allowed to be grown next to watercourses.

5.3 Conclusion

Improving the nutrient-use, environmental and land-use efficiency of miscanthus cultivation is due to various environmental and economic reasons a crucial point for the successful integration of miscanthus in agricultural production systems.

Collar propagation is hereby a promising way to improve the environmental efficiency of miscanthus production. As collar harvest is conducted without soil disturbance, the risk of soil erosion and CO_2-losses – and therefore also the risk of negative effects on soil quality – is reduced. Besides propagation, also the environmental performance of miscanthus removal was analysed in this study. It was shown that soil N_{min} increases after miscanthus removal but on a low level, indicating that no flush of nitrogen has to be expected thus significantly reducing the risk of nitrogen losses such as nitrate leaching.

Closely related to the environmental performance is the nutrient-use efficiency, as fertilization could lead to negative environmental impacts. In addition, the use of fertilizers also increases the production costs. Therefore, a high nutrient-use efficiency is desirable from an environmental as well as economic point of view. Through this study it was demonstrated that if miscanthus is utilized as biogas substrate, a delayed harvest to mid-October maximises nutrient-use efficiency and, additionally, enhances its green-cut resilience.

High biomass and methane yields are key in order to achieve a high land-use efficiency. Hereby, a vegetative propagation of miscanthus via collars is an interesting option, as the harvest is less destructive and rhizomes remain in the mother field. In contrast to the propagation via rhizomes, this allows resprouting of the collar mother field and thus ensures a yield. Additionally, in the establishment year miscanthus could be grown together with maize to overcome the yield gap of the first year through high yielding maize. For use as biogas substrate, miscanthus should be harvested in Germany in mid-October. This harvest date ensures a high silage quality resulting in high methane yields. Additionally, it secures a long lifetime of the miscanthus stand and thus a high land-use efficiency. Breeding miscanthus genotypes with a higher leaf proportion could improve land-use efficiency even further, as such genotypes have a higher substrate-specific methane yield. In order to integrate miscanthus in a crop rotation it is crucial to minimize negative effects on the following crop. In this context, maize was shown to be the most suitable crop to be grown afterwards.

In conclusion, this study showed that there are several opportunities to significantly increase the nutrient-use, environmental and land-use efficiency of miscanthus biomass production.

These results are therefore an important step for the successful implementation of environmentally and economically sustainable miscanthus based value chains in practice.

5.4 References

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7 Curriculum Vitae

Personal data

Name: Anja Maritta Mangold

Date of birth: 07.12.1990
Place of birth: Laichingen

Nationality: German

Education

06/2016 - 09/2019	**Doctoral student in agricultural science at the University of Hohenheim** Department of biobased products and energy crops PhD thesis: *How can miscanthus be integrated most efficiently into agricultural production systems?*
10/2015 - 09/2016	**Masterthesis** *Alternative Biogassubstrate zu Mais - ein Vergleich zwischen Ganzpflanzensilage aus Triticale und einem Triticale / Roggen-Gemisch*
10/2014 - 09/2016	**Master programme University of Hohenheim** *Biobased products and bioenergy* Master of Science
06/2014 - 09/2014	**Bachelorthesis** *Auswirkungen des EEG 2014 auf die Biogas-branche in Deutschland*
10/2011 - 09/2014	**Bachelor programme University of Hohenheim** *Biobased products and bioenergy* Bachelor of Science
09/2001 - 06/2010	**Albert-Schweizer-Gymnasium Laichingen** *General qualification for university entrance*

Professional career, practical courses, expericences

06/2016 - 09/2019	**Research assistant University of Hohenheim** Department of biobased products and energy crops
07/2015 - 05/2016	**Student employee Festool GmbH – environmental management** Field of activity: support in day-to-day business in following areas: DIN 14001, REACh, RoHS, WEEE
04/2013 - 06/2015	**Student employee Ingenieurbüro Vaßen** Field of activity: research and support in emission control (noise forecast, odor-emission forecast, stack height calculation) Support in creation of permit applications Renewable-energy-law: data acquisition of client base and support in verification of biogas plants
03/2013 - 03/2013	**Internship at the Ingenieurbüro Vaßen for four weeks**
10/2012 - 08/2013	**Voluntary participation at the `Humboldt-Reloaded Project`** Topic: energy storage
09/2010 - 08/2011	**Voluntary Internship** Geriatric rehab hospital Ehingen
Several times a week	Support at the familial agricultural enterprise

Skills

Languages	German (native); English (fluent)
Certificate	Certificate of competence in crop protection
Computing	MS Office

Conference contributions

03/2019	MISCOMAR Conference in Katowice (Polen) Presentation: Miscanthus biomass conversion via anaerobic digestion and combustion
11/2018	End-Term Meeting of FACCE SURPLUS 1st Call in Düsseldorf (Germany) Poster: Miscanthus for biogas production: Optimal harvest date, ensiling and effect of leaf:stem ratio on methane yield
05/2018	26th European Biomass Conference in Copenhagen (Denmark) Presentation: Miscanthus for biogas production: Optimal harvest date and ensiling
09/2017	60. Pflanzenbautagung in Witzenhausen (Germany) Poster: Neue Miscanthusgenotypen für eine umweltverträglichere Biogasproduktion
09/2017	Second Bioeconomy Congress in Hohenheim (Germany) Presentation: “Novel miscanthus genotypes for a more environmentally benign biogas production”

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von Cossel, M.; **Mangold, A**.; Iqbal, Y.; Hartung, J.; Lewandowski, I.; Kiesel, A. (2019): How to Generate Yield in the First Year - A Three-Year Experiment on Miscanthus (*Miscanthus x giganteus* (Greef et Deuter)) Establishment under Maize (*Zea mays* L.). *Agronomy*, **9**, 237. Doi: 10.3390/agronomy9050237.

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Hohenheim, den 01.12.2019

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